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The effect of stimulus location and initial posture on the kinematics of the nociceptive withdrawal reflex of the tail in intact and unanaesthetized rats

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The Effect of Stimulus Location and Initial Posture on the Kinematics of the Nociceptive
Withdrawal Reflex of the Tail in Intact and Unanaesthetized Rats

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A thesis submitted to the Graduate Faculty of

JAMES MADISON UNIVERSITY

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Abstract

The nociceptive withdrawal reflex (NWR) is a protective reflex that allows mammals to avoid noxious stimuli by withdrawing the affected area of the body away from the stimulus. Although previous studies on non-human mammals showed that the NWR depends on stimulus location and initial posture, these studies were performed on spinalized or anaesthetized animals, which may yield results differing from intact animals. Therefore, the purpose of this study was to determine whether the kinematics of the NWR of the tail depends on stimulus location and initial posture in *intact and unanesthetized* animals. The NWR was evoked by heat applied to the lateral aspect of multiple locations along the length of the tail in *intact and unanesthetized* rats. The tail was either initially straightened or rotated/curved. Movement in the horizontal plane was recorded using high speed video. Movement consisted of only two components: rotation around the base of tail and a local bend near the stimulus location, one location rostral to stimulus location. When the tail was initially rotated, but not curved, the direction of tail base rotation reversed; that is, when the tail was straight the tail base rotated away from the stimulus, but when the tail was initially rotated, the tail base rotated into the stimulus. These results suggest the rat uses a simple strategy to withdraw from localized noxious stimuli. Following stimulation, muscle(s) apply rostral-lateral force directly to the segment being stimulated, which results in an active local bend, passive rotation of the more proximal tail base and passive “whipping” of the distal regions of the tail. When the initial posture of the tail is altered by rotating the tail to the side, the reflex movement around the tail base reverses, thus moving the tail into the stimulus but away from the body. The change in direction may arise passively from the altered posture, further

simplifying control. Taken together, although the kinematics of the tail movement is complex, these results suggest that the rat uses a simple active and passive kinetic strategy to effectively withdraw its tail from noxious stimuli under various postural conditions.

Introduction

Movements often need to be accurate, fast, or coordinated. However, movement planning is computationally intensive (Franklin and Wolpert, 2011) and even for simple movements it is not possible to be both accurate and fast (Fitts, 1954). These observations raise a question; why is movement planning complex? Beyond the multiple, non-linear differential equations that must be solved (Hollerbach and Flash, 1982) by the central nervous system (CNS) for multi-joint movement, a major computational barrier to movement planning and execution are two “degree-of-freedom (DOF) problems” (Franklin and Wolpert, 2011). The first is simple; Humans have over 100 joints in their body, a large portion of which are required for even simple movements such as standing and walking. Second, we have redundant joints; that is, more joints than necessary. For example, for a human to position a pencil in space at any location with any angle of the pencil, six degrees of freedom (three to position the pencil in space and three to orient the pencil) must be specified. However, human arms have seven degrees-of-freedom (three at the shoulder, one at elbow, and three at the wrist). Consequently, the nervous system, beyond calculating neural command signals, must also select from an infinite number of redundant but equivalent combinations (Bernstein, 1967).

There are three potential solutions to the DOF problems. First, kinematic (movement) synergies (d’Avella et al., 2003; Tagliabue et al., 2015) can constrain two or more joints to operate as a single joint. As long as there are redundant DOFs, all movements can still be created (Rosenbaum, 2009). For example, for reaching movements, joint rotations

around the elbow and shoulder can be tightly correlated, thereby decreasing the DOF from two to one (Bernstein, 1967).

The second solution is to re-use higher level motor commands. For example, handwriting with the non-dominant hand was similar to that of the dominant hand, suggesting that the same motor program was used for both arms (Raibert, 1977). Thus, with this solution different movements would share characteristics such as path or direction.

The third solution is simply to select preferred movement patterns, thereby decreasing the number of choices considered by the CNS. For example, in the spinalized rat, heat stimuli delivered to spots distributed circumferential around the tail resulted in only two possible movements – ventral-left and ventral-right (Cleland and Bauer, 2002), even though the rat was capable of moving its tail in any direction.

While mammalian limbs are somewhat redundant, other body parts in animals have a great many redundant DOF, known as hyper-redundancy (Kang et al., 2011). The control of these structures present a significant computational challenge for nervous systems, however they provide ideal experimental models for the exploration of strategies designed to simplify neural control and movement planning (Gutfrend et al., 1996). For example, the octopus arm, which is composed of muscle but no joints, has an infinite number of DOF because it can bend anywhere along its length (Yekutieli et al., 2002). In rats, the structure with the greatest number of DOFs is the tail, which has 28 vertebral

joints (Brink and Pfaff, 1980) and therefore potentially 84 degrees of freedom (flexion - extension, abduction – adduction, and rotation; Monheit and Badler, 1990).

The overarching goal of the experiments described below is to identify the movement strategies used by the rat to control its tail during a simple reflex, thereby providing insight into whether kinematic synergies, re-used motor commands or preferred movement patterns are used to simplify the computational problem of moving the tail.

The Nociceptive Withdrawal Reflex

The nociceptive withdrawal reflex (NWR), first studied by Sherrington in the beginning of the 20th century, consists of flexion movement around the hip, knee and ankle in response to noxious cutaneous stimulation anywhere on the hind limb (Sherrington, 1906). Based on electrical stimulation of peripheral mixed and sensory nerves, Sherrington showed the NWR resulted from both excitation of flexor and inhibition of extensor muscles throughout the limb (Sherrington, 1910). Consequently, he termed the response “flexion-reflex of the limb” (Sherrington, 1910), though for noxious stimuli the response is now known as the nociceptive withdrawal reflex. The NWR has been proposed to serve a protective function because the pattern of muscle activities, as well as the resulting movement, would be expected to cause the stimulated region of the limb to move away from the noxious stimulus (Sherrington, 1910).

Sensory Mechanisms

Diverse noxious natural and artificial stimuli can evoke or facilitate the NWR, including heat (Morch et al., 2006), mechanical (Schouenborg and Kallimaki, 1990), electrical

(Andersen et al., 1999) and chemical (Gilchrist et al., 1996) stimuli. Two classes of nociceptors detect these noxious stimuli: A δ and C fibers (Dubin and Patapoutian, 2010). A δ - fiber nociceptors have small receptive fields and faster conduction velocity due to myelination around the afferent axons (Treede et al., 1995). In contrast, C - fiber nociceptors have large receptive fields and slower conduction velocity due to their lack of myelination (Dubin and Patapoutian, 2010; Yeomans and Proudfit, 1996). The adequate stimuli also differ; A δ nociceptors respond largely to either temperature (typically hot) or pressure, while C – fiber nociceptors respond to heat, pressure and chemical stimuli (Van Hees and Gybels, 1981; Cain et al., 2001). Centrally, A δ and C fibers also differ in their central actions (Dubin and Patapoutian, 2010), neurochemistry (Basbaum et al., 2009) and perceptions evoked (Dubin and Patapoutian, 2010).

Both A δ and C fiber nociceptors are present in the tail of the rat. Although the number of studies is limited, noxious mechanical stimuli excite both A δ and C fiber nociceptors (Handwerker et al., 1987). Similarly, heat stimuli applied to the tail also excite both A δ and C fibers, although thresholds differ; stimuli higher than 37°C are sufficient for C – fiber but greater than 50°C is required for A δ - fiber nociceptors (Fleischer et al., 1983; Handwerker et al., 1987).

Spinal Mechanisms

The spinal cord is sufficient to mediate the NWR, as demonstrated by the occurrence of the NWR in spinalized animals (Cleland and Bauer, 2002; Schouenborg et al., 1992) and humans with spinal cord injuries (Kim et al., 2007). The NWR is therefore mediated by

interneuronal circuits within the spinal cord, which connect the noxious sensory information that enters through the dorsal roots with ventral motoneurons. The minimum number of intervening synapses (Eccles and Lundberg, 1959) is two (for excitation to motoneurons) or three (for inhibition to extensor motoneurons), although there are likely to be alternative pathways that involve numerous synaptic connections (Eccles and Lundberg, 1959).

The spinal interneurons that mediate the NWR are located throughout Rexed laminae I-IV, located in the dorsal horn of the gray matter of the spinal cord (Grossman et al., 1982), which are known to mediate nociceptive processing (Willis and Coggeshall, 1978). Anatomical studies tracing afferent pathways with horseradish peroxidase (HRP) show afferents terminating in sacral 4 (S4), coccygeal 1, 2 and 3 (Co1, Co2, and Co3) (Grossman et al., 1982). Neurons that respond to noxious stimulation and may mediate the NWR have been identified predominantly in lamina II - IV for mechanical and laminae IV - VI for heat stimuli (Cervero et al., 1988). However, nociceptive spinal interneurons may contribute to ascending projection but not spinal reflexes. Therefore, Schouenborg et al. (1995) specifically sought extracellular recordings from spinal interneurons whose patterns of activity matched previously described patterns of activity of muscles responding during the NWR. These spinal interneurons encode specific information on the NWR of a single muscle (Schouenborg et al., 1995). Morgan (1998) went a step further and sought spinal interneurons while simultaneously evoking the NWR in lightly anesthetized animals, similarly demonstrating that candidate interneurons could be found in laminae I, II, and V.

As with nociceptors, there is a similar limitation in the number of studies of spinal interneurons that mediate the tail NWR. Cervero et al. (1988) showed that the activity of both nociceptive-specific neurons (only responding to nociceptive input) and to a lesser degree multi-receptive neurons (responding to both nociceptive and non-nociceptive input) in laminae I, II and V coincided with the onset of the tail NWR. Also, Douglass and Carstens (1997) showed that spinal interneurons had tail cutaneous receptive fields that extended bilaterally.

Supraspinal Mechanisms

Although the spinal cord is sufficient to mediate the NWR (Sherrington, 1910), there is extensive evidence in animals that descending pathways from the brain can alter the presence, direction, and strength of the NWR (Eccles and Lundberg, 1959; Cleland and Bauer, 2002; Dimitrijevic and Nathan, 1970). For example, the NWR was present in spinalized but absent in decerebrated (transection through the brainstem) animals (Eccles and Lundberg, 1959; Holmqvist and Lundberg, 1961). Spinalization also alters the direction of the movement of the tail; intact rats moved their tail dorsally but spinalized rats moved their tail ventrally (Cleland and Bauer, 2002). Activation of brainstem locations by pressure injection of neurochemicals can either increase or decrease the strength of the NWR (Gebhart, 2004).

Similarly, humans with spinal cord injuries exhibit a stronger and longer NWR (Dimitrijevic and Nathan, 1970) with larger reflex receptive fields (Andersen et al., 2004) than normal patients. Spinal cord injured patients also displayed a simpler form of the

NWR, without the presence of a “local sign” (small differences in the magnitude of muscle responses; Schmit et al., 2003).

In order to identify the loci of the higher neural structures that can influence the NWR, Holmqvist and Lundberg (1961) studied on decerebrated animals. The authors made progressive transections of the brainstem starting rostrally (upper pons) and then working caudally (to the lower medulla). Inhibition of extensor motoneurons was released when the lesion reached the pons. However, excitation of flexor motoneurons was released only once the lesion reached the medulla. Their results demonstrated that animals with an intact brainstem did not exhibit the NWR, but that descending afferents on flexor and extensor motoneurons during the NWR are mediated at least in part by different regions of the brainstem.

The descending pathways that could mediate the effects on the NWR are numerous. Based on studies in which the NWR was used as a proxy to study pain perception in animals, descending pathways originating from the dorsolateral pontine tegmentum and rostral ventromedial medulla were shown to both facilitate and inhibit spinal nociception. The differential effects depended on the neurotransmitter released, with serotonergic pathways inhibiting and noradrenergic pathways exciting the NWR (Gebhart, 2004).

Studies focused on the descending control of movement have implicated the vestibulospinal and reticulospinal pathways that descend in the medial spinal tracts.

The vestibulospinal pathway, originating in the lateral vestibular nucleus and integrating vestibular feedback, was shown to preferentially excite extensor motoneurons (Wilson and Yoshida, 1969). In contrast, reticulospinal pathways have been shown to excite flexor motoneurons (Schwindt, 1981).

Supraspinal Control: Descending modulation

Descending pathways can influence the spinal NWR through two mechanisms: long-loop reflexes and modulation. Modulation of spinal reflexes differs from reflex activation in that modulation does not directly influence motor output, but rather alters the strength or other characteristics of the spinal reflex. Typically, descending pathways are tonically (continuously) active, thereby setting the strength of the reflex. When an inhibitory descending pathway is eliminated by spinal transection, the reflex would become stronger; if the descending pathway were excitatory the reflex would become weaker.

There is extensive evidence, mainly from studies on nociception, that brainstem pathways tonically both excite and inhibit the NWR, although the balance is tipped toward inhibition since chemical blockade in normal animals results in an increase in pain sensitivity (Gebhart and Proudfit, 2005). One of the three descending pathways implicated in the modulation of nociception arises in the dorsolateral pontine tegmentum (DPT). The DPT projects to the dorsal horn of the spinal cord and releases serotonin which facilitates nociception. In contrast, the descending pathway from the rostral ventromedial medulla releases norepinephrine to inhibit spinal nociception (Gebhart and Proudfit, 2005; Gebhart, 2004; Leonard, 1998). The third possible descending pathway

originates in the nuclei reticularis gigantocellularis and gigantocellularis pars alpha, which have both excitatory and inhibitory modulatory actions on the NWR. Since most of these studies used the NWR of the foot to measure nociception, it is likely their results are applicable to the tail NWR.

Supraspinal Control: Long-loop reflex

The second supraspinal mechanism that could influence the NWR is the long-loop reflex (Macefield, 2009). The long-loop reflex is similar to a spinal reflex except the pathway is not confined to the spinal cord but includes supraspinal structures (Christensen et al., 1999), typically the cerebral cortex (Lee and Tatton, 1975). For example, in the best studied long-loop reflex, muscle spindle afferents enter the spinal cord and bifurcate, with one branch progressing through the spinal cord to synapse directly on to motoneurons (the stretch reflex), while the other branch ascends the spinal cord to the dorsal column nuclei. Subsequently, the afferent signal is conveyed to the thalamus, cerebral cortex and then back down through the corticospinal pathway to the spinal cord to constitute the reflex pathway. Consequently, the latency of the long-loop reflex exceeds that of the spinal reflex (Matthews, 1991). There are two lines of evidence that the NWR includes a long-loop component: latency and neural recording.

Noxious electrical stimulation of the hind limb evokes two electromyography (EMG) responses with separate latencies. The first EMG burst, RII, consists of a short latency activity between the ranges of 56 – 65 ms, while the second EMG burst, RIII, has a longer latency time of 85 – 180 ms (Sandrini et al., 2005; Meinck et al., 1985). Due to the

differences in latencies, it was suggested that the RII component is a spinal NWR reflex and the RIII component is a long-loop NWR reflex. However, latency alone is not sufficient to establish path, since both short and long-loop reflexes can be present in a spinal reflex (Sandrini et al., 2005; Tracey et al., 1980).

The second line of evidence is that the neurons in the brainstem, specifically the rostral ventromedial medulla, known to influence spinal NWR pathways, increase their firing rate immediately after a noxious stimulus but still before the reflex response. Further, the time course of neural activity corresponds to the time course of the NWR muscle activity (Devonshire et al., 2015).

Dependence of the nociceptive withdrawal reflex on stimulus location

Studies on the effect of stimulus location on the NWR have identified three alternative movement strategies: independent of stimulus location (Sherrington, 1910), dependent on stimulus location (Schouenborg and Kalliomaki, 1990), and a hybrid strategy (Cleland and Bauer, 2002). In a dependent strategy, changes in stimulus location necessarily result in a change in the direction or magnitude of the withdrawal response, thereby potentially providing a better match between the location of the stimulus and direction of the response. In an independent strategy, responses are quantized; there are only one or a few possible responses. The potential advantage of dependence is that with fewer alternatives, response latency may be shorter choice (Schmidt, 1982), thereby minimizing injury. Hybrid strategies, which combine dependent and independent strategies, may combine the advantages of the individual strategies.

Supporting an independent strategy, Sherrington found that evoking the NWR by stimulation of various afferent nerves of the hind limb in decerebrated animals produced predominately similar patterns of muscle activation and relaxation, in which flexor muscles were excited and extensor muscles were inhibited (Sherrington, 1910). Although there were differences in the magnitude of muscle responses, which were termed “local sign”, Sherrington still considered the NWR to be a single movement because muscle activity did not switch from excitation to inhibition or inhibition to excitation (Creed and Sherrington, 1926). Similar results, in which stimulus location had little or no effect on the NWR, were reported for heat stimulation of the lower limbs of humans (Morch et al., 2006) and electrical stimulation of the lower limbs of spinal cord injured humans (Schmit et al., 2003).

In contrast, other studies have suggested that the NWR is depend on stimulation location. Although Sherrington championed an independent strategy, he recognized local sign in which the relative strength of activation of pairs of flexor muscles could vary by a factor of five (Creed and Sherrington, 1926). Hagbarth (1952) further showed that changing the location of the stimulus could reverse excitation and inhibition in flexors and extensor muscles by stimulating small branches of cutaneous nerves. Further, stimulation of the skin innervated by each sub-branch evoked the same pattern of either flexor excitation/extensor inhibition for some regions and flexor inhibition/extensor excitation for other skin regions. Hagbarth (1952) also observed that for all skin regions the pattern of muscle activation would be expected to move the skin away from the source of stimulation.

Subsequent studies in lightly anesthetized or spinalized animals, and humans, revealed that different muscles in the hind limb are excited or inhibited by different regions of skin. For example, dorsi-flexor muscles in the foot are predominantly excited by plantar stimulation of the toes while plantar flexors are excited by stimulation of the heel (Schouenborg and Kalliomaki, 1990). Similar results were obtained in spinalized rats (Schouenborg et al., 1990), cats (Levinsson et al., 1999), and humans (Anderson et al. 1999, Sonnenborg et al., 2000). The authors in all of these studies emphasized that the observed patterns of muscle EMG would be expected to move the stimulated skin directly away from the location of the stimulus.

It has also been suggested (Cleland and Bauer 2002) that animals combine these two strategies: independence of stimulus location and dependence of stimulus location. Whereas Sherrington (1910) supported independence, his published results revealed that stimulation throughout the leg led uniformly to activation of hip flexors (independence), while only stimuli to the foot and lower leg activated ankle flexors (dependence). Similar results have been obtained in cats (Schouenborg et al., 1994) and humans (Anderson et al., 1999).

The effect of stimulus location on the NWR has been predominantly examined by EMG rather than movement (Levinsson et al., 1999; Schouenborg and Kalliomaki, 1990; Morch et al., 2006). Although isometric joint torque can be calculated from EMG for a single joint (Rugy et al., 2012), determining three-dimensional movement for a multi-joint limb is highly inaccurate for several reasons. First, the force produced by a muscle

depends non-linearly on muscle length and velocity (Maganaris, 2001). Second, the angle of force depends on joint angle (Johnson et al., 2008). Finally, because EMG magnitude depends on position of electrodes (Loeb and Gans, 1986), the effects of different muscles are not easily compared.

Therefore, to understand how multi-joint limb movement depends on stimulus location, it is necessary to measure actual movement; however few studies have done so. In humans, skin stimulation over the foot evoked joint rotations that moved the skin away from the stimulus, although the magnitude of the response was small ($< 8^\circ$) and only seen clearly with artificial electrical stimulation (Anderson et al., 1999). In the upper limb, stimuli delivered to Digits I and V only evoked responses that differed by 30° , relative to 360° . Clarke and Harris (2004) suggested that in lightly anesthetized rabbits, stimulus-evoked rotation around the ankle moved the skin away from the stimulus. In both spinalized and intact rats, the changes in the circumferential location of the stimulus had only modest effects on response direction (Cleland and Bauer, 2002; Bence, 2010). Taken together, although the NWR depends on stimulus location, only small differences in magnitude and direction of movement have been reported.

Dependence of the nociceptive withdrawal reflex on the initial posture of the limb

In order to avoid potentially harmful stimuli, animals need to withdraw their body parts effectively; however, they also need to simultaneously maintain their balance during the reflex withdrawal. In order to maintain balance during the NWR, either a second

“balance stabilizing” reflex can be simultaneously evoked or the NWR reflex can be adapted to the circumstances.

For example, Sherrington (1910) showed that noxious stimulation, which evoked flexion in the stimulated limb, also evoked extension in the opposite limb, which he termed the crossed extension reflex (Sherrington, 1910). When the foot is lifted during the NWR, the weight of the body is shifted solely to the opposite leg. The apparent purpose of the crossed extension reflex is to maintain balance by increasing contraction of extensors in the opposite leg to counter the increased load (Decchi et al., 1997).

Another strategy is to directly adjust the NWR. Extension around the hip in spinalized human patients (Kim et al., 2007), flexion of the upper arm in intact humans, (Peterson et al., 2013) and extension of the knee in spinalized cats (Baxendale and Farrell, 1980) all increased the strength of the NWR. These effects are likely to have arisen from modulation by muscle proprioceptors (Baxendale and Farrell, 1980, Kim et al., 2007, Peterson et al., 2013). The functional implications of these adjustments, however, are less clear.

Since there are only a few studies on the effect of initial posture on the NWR and only one in non-human animals, which used spinalized animals, our laboratory conducted studies of the effect of initial hind limb posture on the direction of the limb NWR in intact and unanesthetized rats. The results from our laboratory demonstrated that the NWR movement direction, magnitude, and foot angle were strongly influenced by the

location of foot immediately prior to stimulation (Chrzan, 2013; Seamon, 2015). If the foot was initially rostral, it was moved caudally, but if initially caudal moved rostrally. A similar pattern occurred in the lateral-medial axis. Thus, the adaptation of the NWR appears to result in increased stability.

Specific Aims

Previous studies have begun to determine how the NWR depends on both stimulus location and initial posture, however few studies have been conducted in non-human animals and those that have been were done in spinalized or anesthetized animals. Further, typically EMG rather than movement was recorded. Consequently, the two specific aims of this study were (1) to determine the influence of stimulus location on the NWR and (2) the effect of initial posture on the movement of the NWR in *intact and unanaesthetized* rats. The tail was studied because its hyper-redundant DOF provides an ideal model system for exploring kinematic strategies designed to simplify the neural control and movement. In particular, kinematic synergies, re-use of motor commands and preferred movements may potentially contribute to the strategy and patterns of tail movement in the NWR.

Methods

Animals

Male Sprague Dawley rats (n=49; bred in house from rats obtained from Harlan, Indianapolis, IN; 108 ± 27 Standard deviation (SD) days, range 72 - 239 days) with a mean weight of 396.5 ± 87 SD g (range 136 - 625) were housed in cages with unlimited water and food and exposed to 12 hours light and dark rotations (light 8:00 - 20:00). The room temperature was maintained between 68 - 72°F and humidity between 55 - 65 %. At the end of the experiment, rats were killed first by exposure to carbon dioxide and secondarily by cervical dislocation. The Animal Care and Used Protocol (A09-13) was approved by the James Madison University Institutional Animal Care and Use Committee.

General methods

Rats were restrained inside a transparent acrylic cylinder (6.5 cm inner diameter), which was lined with a sock to provide a dark, soft environment. Tail length was manually measured with ruler from anus to the tip of the tail in order to establish stimulus and tracking locations. Twelve circular locations (2-5 mm in diameter) were marked with even spacing on the dorsal surface of the tail (Fig 1) with a fine black permanent marker (Ultra fine point sharpie, Sharpie, Downers Grove, IL). A thirteenth location (#1) was marked equidistance rostral to the most rostral location (#2; Fig 1) to provide an estimate of the origin of rotational movement around the base of the tail. The acrylic cylinder was placed on top of a white foam board platform within a cutout to stabilize the cylinder (Fig

2). Rats were acclimated to the environment for 20 minutes prior to beginning the experiment.

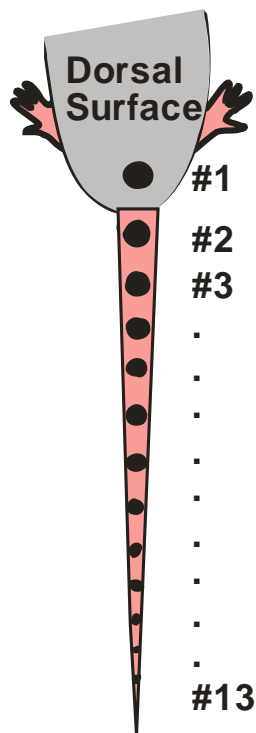


Figure 1. Stimulus and tracking locations. The tail length was measured from anus to the end of tail to mark 12 circular locations (#2- 13) with even spacing on the dorsal surface of rat's tail with a black fine permanent marker to indicate stimulus aiming locations and tracking locations. Location #1 was marked equidistant above the 12 locations to estimate the origin of the base of the tail rotation. Although the left side of twelve locations (#2-13) was stimulated for experiments on the effect of stimulus location, all 13 locations were tracked to determine the tail movement over time.

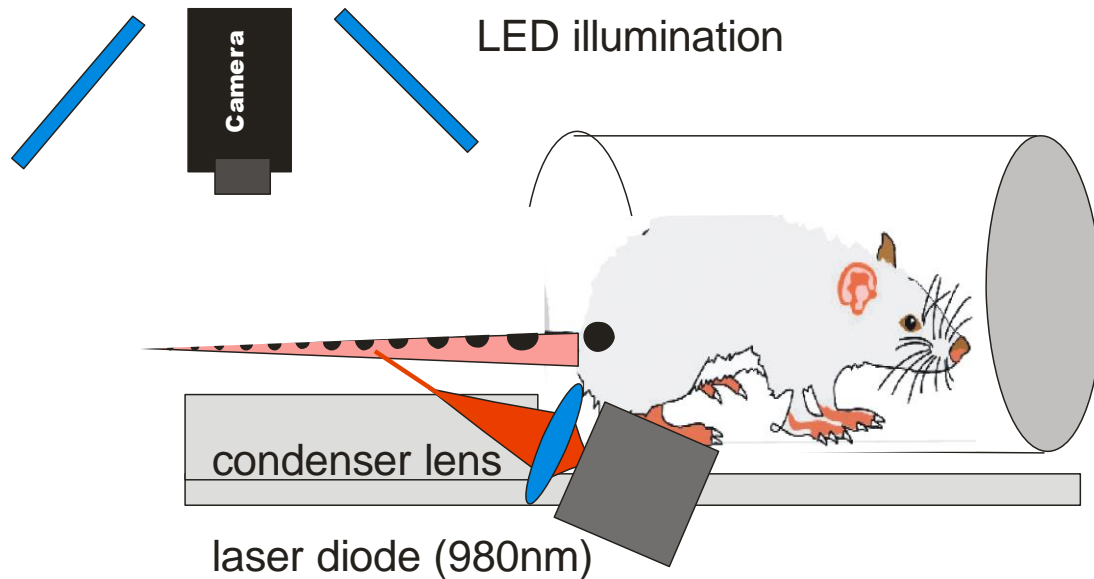


Figure 2. Experimental setup. The rat was restrained horizontally inside of an acrylic cylinder surrounded by a white sock (not shown). The tube was placed on top of a cutout in a white foam board to reduce the movement of the tube and to level the tail with body. The camera was secured perpendicularly above (80 cm) the rat to record the rat's movement in the horizontal plane. Illumination was provided by two LED lights (Fancier LED 1000A, Fotodiox, Waukegan, IL).

A high-speed video camera (NR5-SI, IDT, Tallahassee, FL) with a 50 mm lens (Fujinon, Valhalla, NY) was positioned 80 cm above the rat to record the component of the movement in the horizontal plane. The tail moved predominantly within the horizontal plane, with less than approximately 5% of movements in a primarily vertical direction, which were not recorded. The camera recorded at 650 frames per second and the shutter period was 1/5000 to capture the fast movements of tail. In order to maximize the amount of light entering the lens but also to ensure adequate depth of field, the lens aperture was f/4 (possible range f/1.8-f 22). The video camera was connected to a computer to allow the video to be saved directly to the computer as a series of highest quality jpeg files, each corresponded to single frame of the video.

To provide illumination, two LED lights were placed on both the anterior and posterior sides of the rat at an angle of 45 degrees. LED lights produce virtually no heat, thus minimizing heating of the rat. The lights were on throughout the experiment to minimize providing cues about the stimulus to the rat.

Heat stimuli were delivered to the tail using a 980 nm infrared laser diode (range 0.5 -15 watts; BWF-5, B&Wtek, Newark, DE) focused by a 3" condenser lens (Edmund Optics, Barrington, NJ) to concentrate the light to a 2 mm spot on the tail. The condenser lens was positioned lateral to the rat and the laser's red aiming laser was focused on the lateral side of the tail, aligned with but not overlapping the previously marked 12 locations Fig 2). The angle of the laser in two dimensions was kept approximately constant. The laser was triggered electronically by laser at the onset of the heat stimulus.

Heat stimulation intensity was adjusted to evoke a withdrawal response using a wide range of latencies (0.2 – 35 sec; Only those between 0.5 and 5 sec were analyzed to better match latencies in the posture experiments) for effect of stimulus location experiments (Straight tail; Figs 3 and 4) and latencies between 1 - 3 sec for the effect of initial posture experiments. However, there was no significant difference between latency of straight tail experiments and latencies of curved/ rotated/ combined posture experiments (Fig 5).

Four minute intervals between trials were used to reduce the effects of one trial on the result of the next, which was longer than most previous studies (Campbell et al. (1991) - one minute; Morch et al. (2006) and Romaniello et al. (2002); 10 - 30 seconds. Based on

the Sunkin (2009) who demonstrated that habituation is strongest in the first two trials, results from two “pre-stimuli” were not collected.

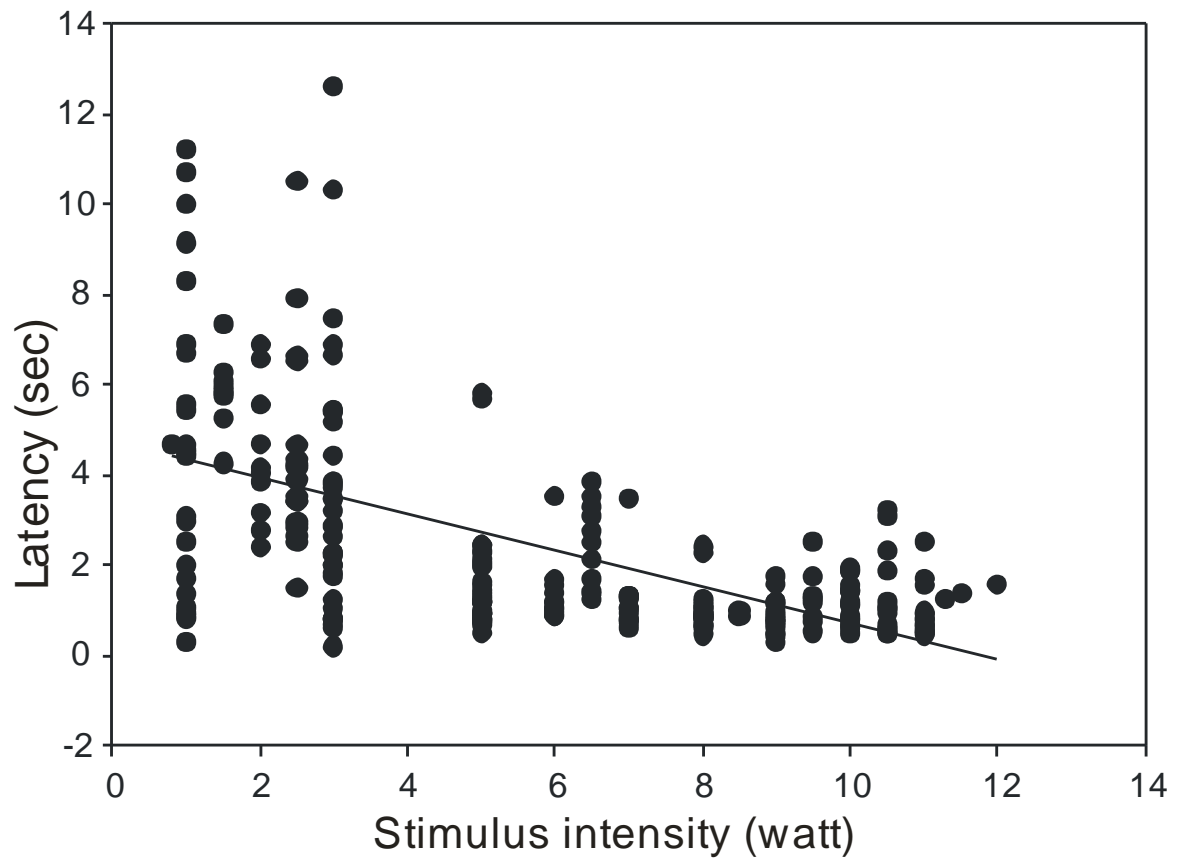


Figure 3. Latency vs. stimulus intensity. For straight tail experiments, Spearman correlation analysis revealed that there was a weak ($r^2=0.38$) but significant ($P< 0.00001$) correlation between stimulus intensity and latency of response across all 12 stimulus locations ($n=247$).

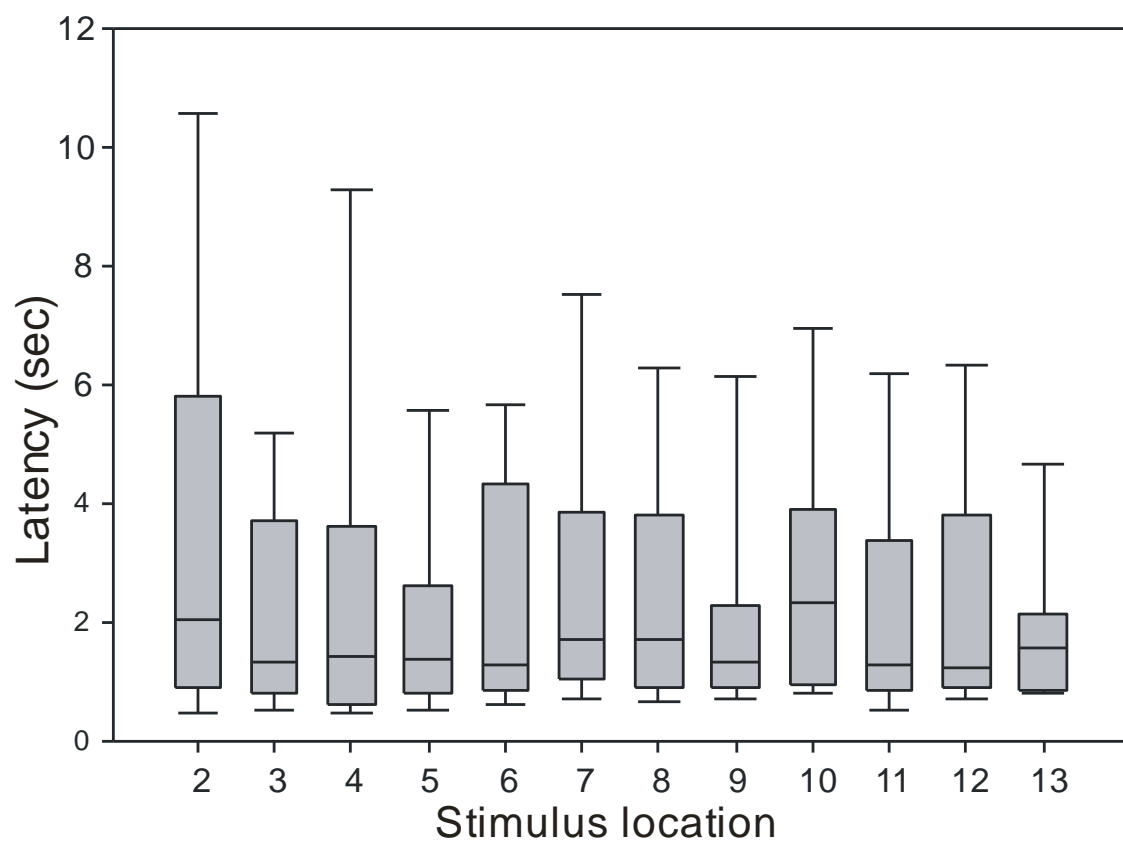


Figure 4. Latency vs. stimulus location from straight tail experiments. The box plot represents latency of response for each of 12 stimulus locations. Latency of response did not depend on stimulus location (Kruskal-Wallis $P=0.93$; $n=247$).

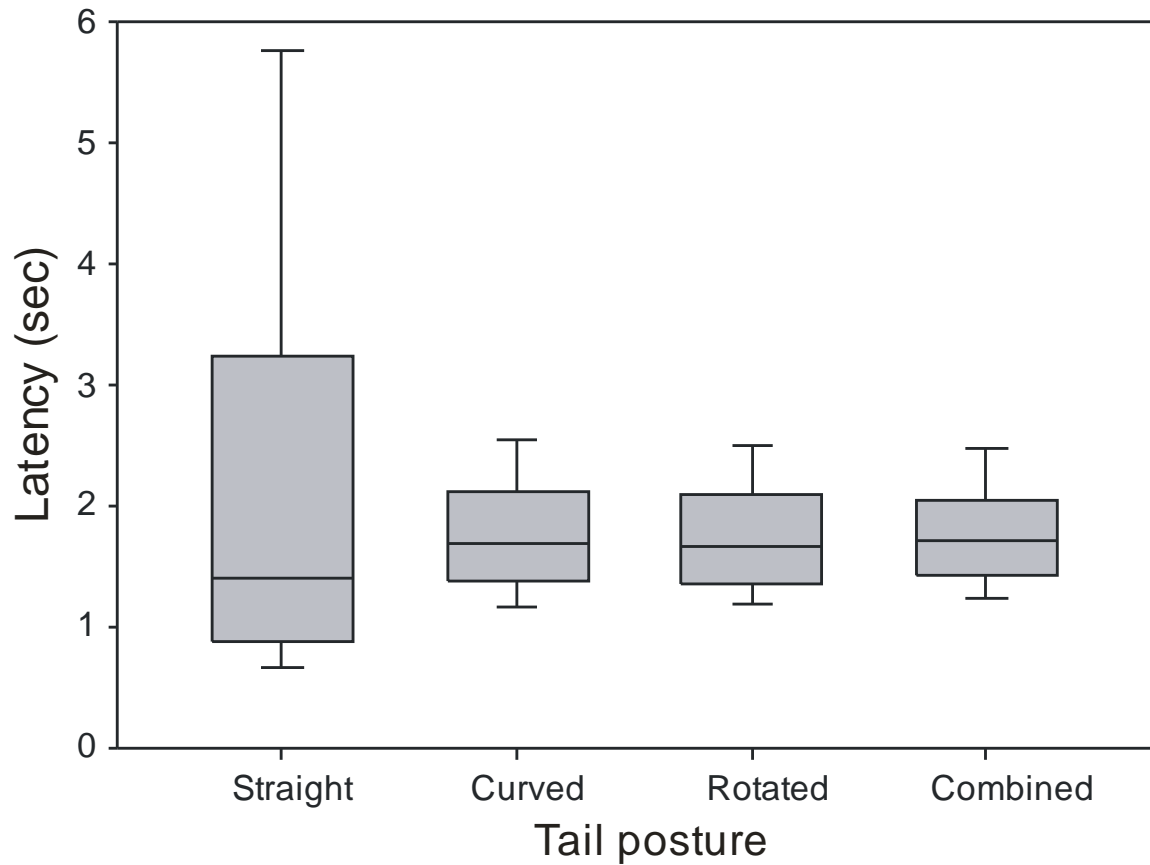


Figure 5. Latency vs. initial posture of the tail. The box plot represents latency of response for each initial tail posture. Latency of response does not depend on initial tail posture (Kruskal-Wallis $P=0.063$; $n=1147$). “Straight” represents straight tail experiments, while each of the three postures includes all trials at all five postures (which includes straight in the posture experiments).

Experimental Protocol

Specific Aim 1: Effect of stimulus location on the NWR. In order to determine the effect of stimulus location and intensity on the NWR, the tail was manually straightened before (5 - 10 sec) each stimulus was delivered. All distal 12 locations (#2 - 13) were stimulated once in random sequence on the lateral aspect of the left side of tail; the sequence was then repeated at a different laser intensity resulting in a total of typically 24 trials per rat. A total 15 of experiments were performed, though about one third of the trials were not analyzed due to extremely small movement, vertical movement or experimental errors.

Specific Aim 2: Effect of initial posture on the NWR. To determine the effect of different initial tail postures on the movement pattern of the NWR, stimuli were delivered to both the left and right side of the tail at three locations; 4, 8, and 12. Three postures of the tail were studied: curved, rotated and combined. A total 30 rats were studied (10 rats for each posture) resulting in a total of 900 trials; all trials were analyzed.

The *curved* posture (Fig 6A) was created by bending the distal tail (~locations 4-13) into a circular curve using a template while keeping the proximal portion of the tail (~locations 1 - 3) straight. Five levels of curvatures were used for each rat, including straight and circular diameters of 64, 32, 16, and 8 cm. The *rotated* posture (Fig 6B) was created by rotating the tail as close as possible to the base (~ locations 1- 3) while keeping the distal portion of the tail (~locations 4 - 13) straight. Five rotations were used for each rat, including straight, 22.5°, 45°, 67.5°, and 90°. The *combined* posture (Fig 6C)

was created by combining identical levels (i.e. 1+1, 2+2, 3+3, 4+4 and 5+5) of curvature and rotation.

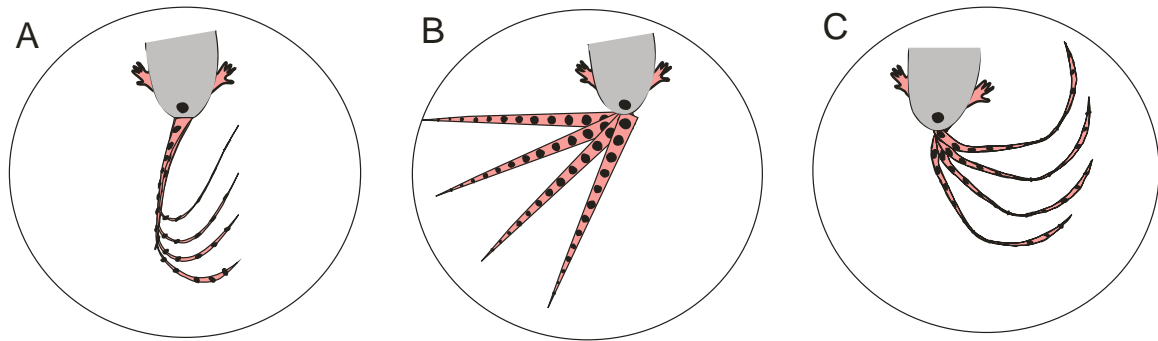


Figure 6. Variation in initial tail posture. Three different initial tail postures were used to determine the relationship between initial posture and the kinematics of the NWR. Five different levels were selected for each posture. (A) Curved posture, in which the tail was curved tail at five different circular diameters, 0 (straight), 8, 16, 32, and 64 cm. (B) Rotated posture, in which the base of the tail was rotated at degree of 0 (straight), 22.5, 45, 67.5, and 90°. (C) Combined posture, in which curved and rotated postures were combined such that the five levels consisted of the combination of the same levels of curvature and rotation.

Data analysis

At the end of each experiment, the recorded videos (individual jpeg file for each frame) were transferred to a computer for further analysis. The most rostral location (location 1) was used as the origin of coordinate system (0, 0) (Fig 8). The Cartesian coordinate system was aligned to the rostral-caudal and lateral-medial axis. The polar coordinate system was ± 180 degrees with 0 degrees aligned with the rostral direction of the rat.

ProAnalyst (Xcitex, Cambridge, MA) was used to track the 13 locations on the tail individually using an automatic tracking system yielding the rostral-caudal/lateral-medial positions in the horizontal plane of all 13 locations over time (Fig 7). Tracked data were

saved in text file and converted from pixels to millimeters based on the image of metric ruler in a calibration frame.

Latency of response relative to stimulus onset was determined as the time following stimulus onset at which the smallest possible movement at any tail location occurred.

Angles of rotation around each of the 12 most proximal locations, including the base of the tail, were calculated as the included angle between the location of interest and the two adjacent locations.

The location of the local bend was obtained in Matlab by spline smoothing the 13 locations for each frame and visually identifying the position, relative to the marked locations, of the peak deflection. The location of local bend identification was done as early as possible after movement began but always within the first 10 frames (1/65 sec).

Data were analyzed in Matlab (Mathworks, Natick, MA) SigmaPlot (Systat Software, San Jose, CA), Oriana (KCS, Wales, UK), and SPSS (IBM, Chicago, IL). For normally distributed data (determined with a Kolmogorov-Smirnov test), and with equal variance for inferential tests, parametric statistics were used; otherwise nonparametric statistics were employed. The exception was circular data, for which circular statistics were employed. More specifically the inferential parametric tests that used were: ANOVA (parametric) and the non-parametric inferential tests Chi², Freidman, Spearman correlation and Kruskal-Wallis. Finally, the circular inferential test used was the Watson-Williams F-test.

Boxplots were used to indicate median with the line in the box, 75th and 25th percentiles indicated with upper and lower box boundaries, 95th and 5th percentile indicated with whiskers. Effect sizes are reported as measures of the magnitude of effect. For Friedman tests, the Kendall's W test was used to determine effect size and for the Chi² test the Cohen's W test was used. Small effect size is 0.10, medium is 0.30 and large effect size is 0.50.

Alpha (α) was set to 0.05 except when correcting for multiple comparisons. For multiple comparisons, Bonferroni corrections were used to reduce the alpha value. Details are given in the figure legend as, for example, MC=4 for multiple four comparisons, $\alpha=0.0125$ for reduced alpha, significant/non-significant to indicate significance based on the reduced alpha value. Multiple comparisons were automatically computed in Post-hoc ANOVAs by a Tukey test. P-values were capped at the low end at <0.00001 .

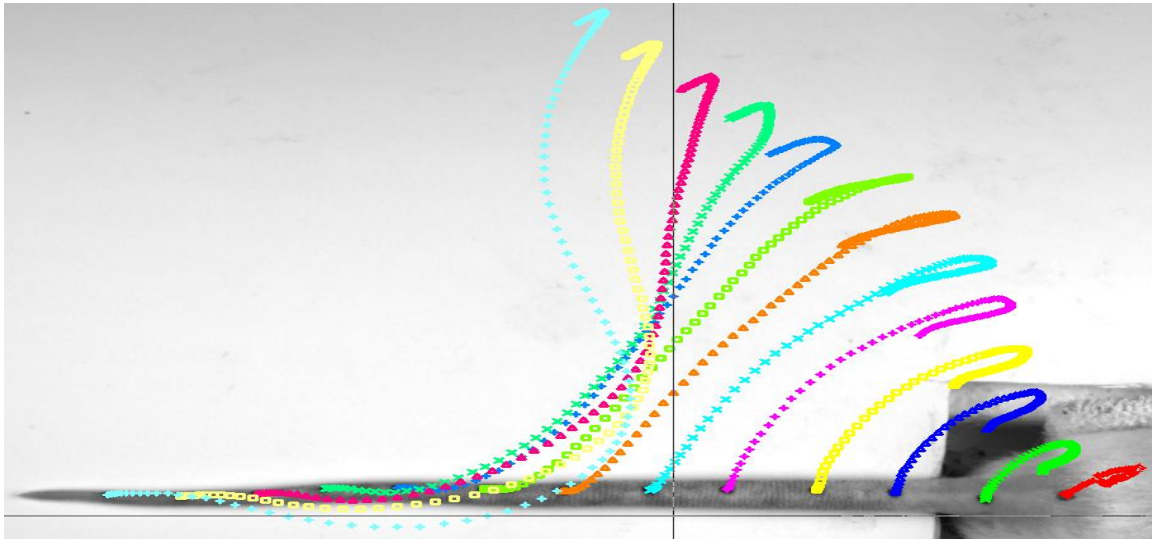


Figure 7. Tracking thirteen locations. The previously marked 13 locations were individually tracked by an automatic tracking system in ProAnalyst to obtain the entire horizontal plane tail movement over the time. Each color represents different locations and the points represent sequential frames.

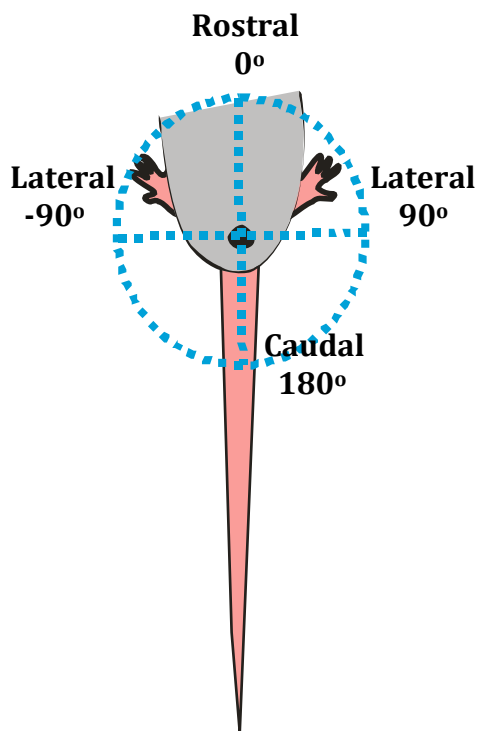


Figure 8. Coordinate systems. A cartesian coordinate system, aligned with the rostral-caudal and lateral-medial axes of the rat, was specified prior to tracking for each trial. Location #1 was used as the origin of coordinate system. The polar coordinates system was ± 180 centered rostrally.

Results

The two specific aims of this study were to determine the effects of stimulus location and initial posture on the kinematics of the NWR. In the first series of experiments (n=15), stimuli were delivered unilaterally to 12 locations distributed evenly along the length of the straight tail. In the second series of experiments (n=30), stimuli were delivered bilaterally to three locations for each of five levels of three different postures.

Straight Tail

Localized heat stimuli delivered to all 12 locations along the length of the tail resulted in reliable (247/247, 100%), short latency (2.02 ± 1.99 SD sec) lateral movements. In most (91.6%), but not all (8.4%) trials, the response was directed away from the stimulated side.

The NWR is comprised kinematically of two components – Tail base rotation and local bend. The tail consists of 28 vertebrae, suggesting at least a similar number of degrees of kinematic DOF in the horizontal plane. However, the observed tail response was largely characterized by only two components of movement: rotation around the base of the tail and local bend (Fig 9). That these are the main components of the NWR is supported by Figure 10, which shows that the largest rotation occurs at the base of the tail and the second largest at a location associated with the local bend; rotations around other locations are all smaller. The second rotation varies with stimulus location in the same way that the local bend varies with stimulus location (Fig 11). Taken together, these

results demonstrate a large decrease (from 28 to 2) in the number of DOF employed by the NWR.

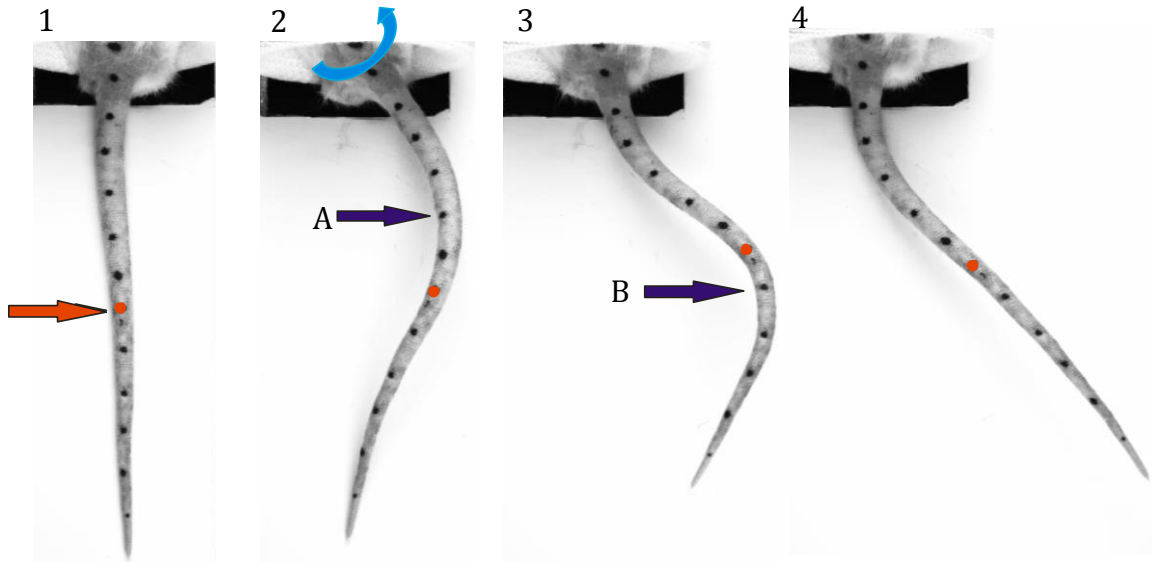


Figure 9. Representative example showing the NWR arises from rotation around the base of the tail and local bend. Four video frames (25ms, 15 frames apart) from a single trial show the movement of the tail in response to heat stimulation at location 8 (indicated by the red arrow in frame 1). In frame 2, the blue arrow illustrates rotation around the base of the tail (rotation of location 2 relative to location 1) and the first purple arrow (A) identifies the location of the local bend. In frame 3, the local bend (B) progresses along the length of the tail as the movement continues. The maximum excursion of the tail is shown in frame 4.

Response direction is invariant: Stimuli were delivered to 12 rostral-caudal locations, raising the question whether similar or different movements were evoked. To determine the effect of stimulus location on the kinematics of the NWR, the response vector was measured for each stimulus location (locations 2-13), which revealed that the response was directed rostral-laterally (mean 63.5°) and was invariant across stimulus location; response did not depend on stimulus location (Fig 12; Watson-Williams $P=0.48$).

Therefore the movements differed, the direction that each stimulated location moved was similar.

The invariant response direction can be explained by a combination of tail base rotation and local bend. The rostral-laterally directed movement could have arisen kinematically

from tail base rotation, local bend or a combination of tail base rotation and local bend.

Figure 10 supports the combined hypothesis by showing that the maximum mean peak rotation around each tail location always occurred at both the tail base and the location of the local bend (Fig 10; ANOVA $P < 0.00001$ (locations 2-10); $P = 0.002$ (location 11); $P = 0.17$ (location 12)).

The local bend match stimulus location. For caudal stimulus locations, the location of the local bend closely matched the location of the stimulus (Kruskal-Wallis $P < 0.00001$; Fig 11). However, the location of the local bend was about one location more rostral than the stimulus location. For more rostral stimulus locations, the local bend leveled out at location 3, possibly due to the increased rigidity of the proximal tail.

Bend progression suggests passive bending distal to the stimulus location. The movement of the tail distal to the local bend could arise either passively from the local bend or actively by direct muscle action on distal segments. If passive, the local bend should progress distally as the movement evolves. For most stimulus locations the local bend progresses distally, supporting the hypothesis that the biomechanical basis for the distal movement is passive (Figs 13 and 14).

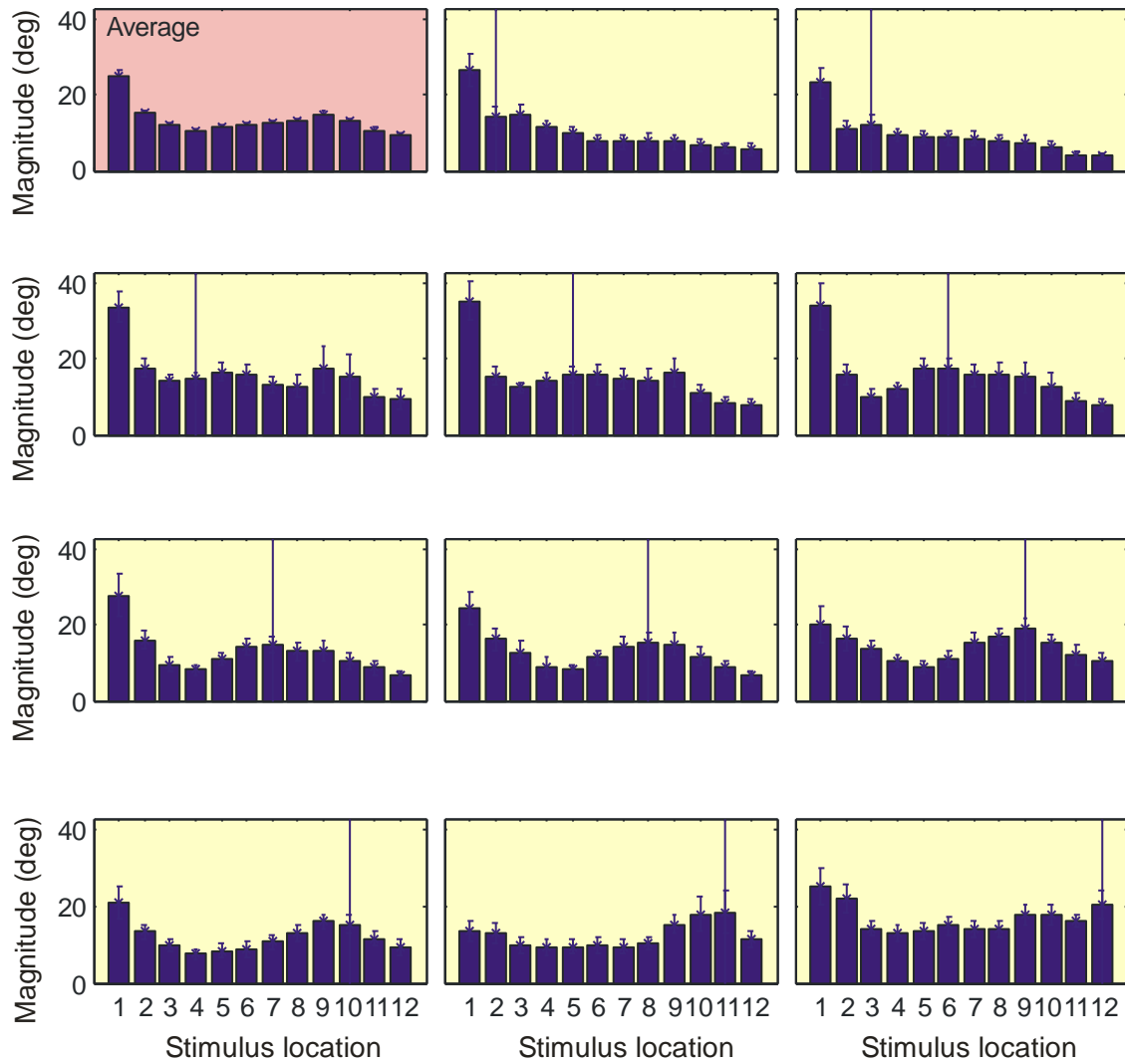


Figure 10. Maximum magnitude of angular rotation vs. stimulus location. Overall (pink background), the largest absolute angular rotation occurred around the base of the tail (location 1) and location 9 (ANOVA $P < 0.00001$), the latter likely corresponding to the local bend. A similar pattern occurred for all 12 stimulus locations except the second peak, or local bend, closely followed stimulus location (ANOVAs $P < 0.00001$ (location 1 – 10); $P = 0.002$ (location 11); $P = 0.17$ (location 12); $MC = 11$; $\alpha = 0.009$).

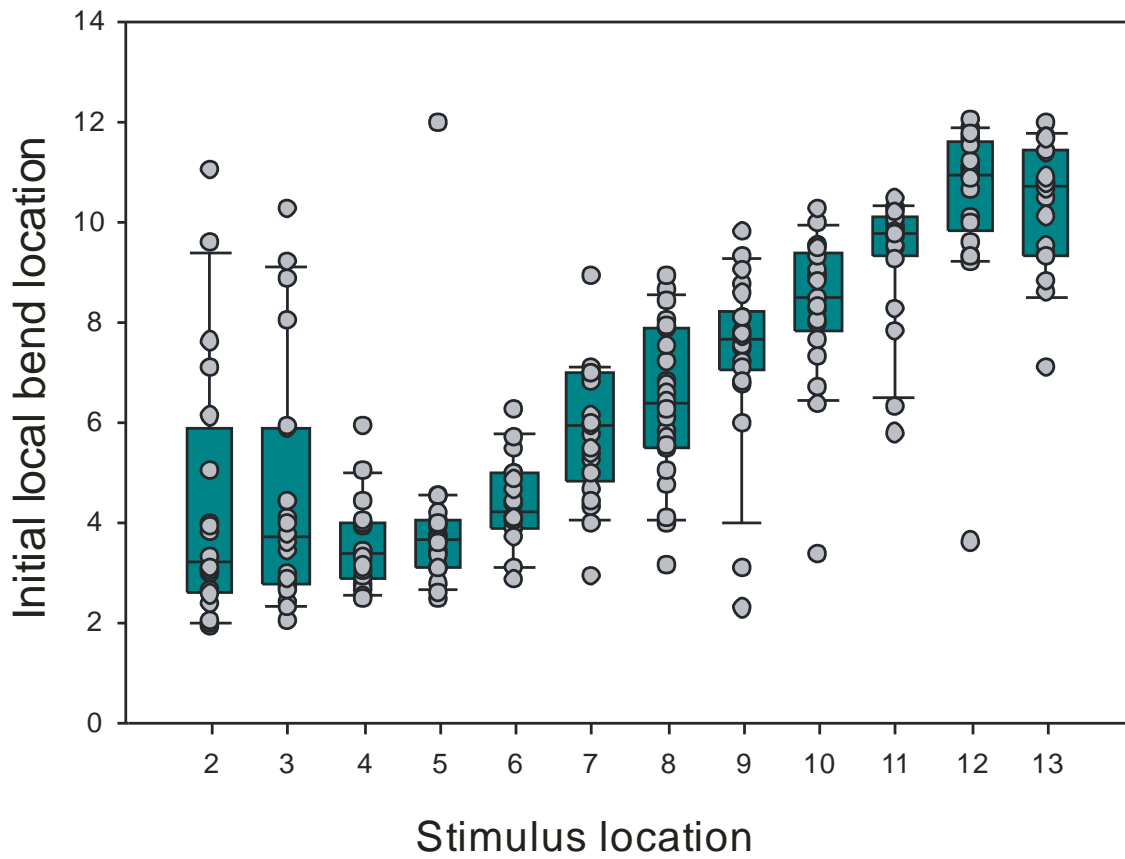


Figure 11. Initial local bend location vs. stimulus location. The dependence of initial (with the first 10 frames following movement onset) local bend location on stimulus location is represented as boxplots and individual data points (gray filled). As the stimulus location was moved distally the initial local bend location moved distally as well, except for proximal locations 2 to 4 (Kruskal–Wallis $P < 0.00001$; $n = 247$, effect size = 0.73). Note the location of the initial bend is typically one location rostral to location of the stimulus. The initial local bend location was only weakly affected, in terms of effect size, by response latency (Multiple linear regression $P < 0.00001$; $n = 247$, effect size only 0.14).

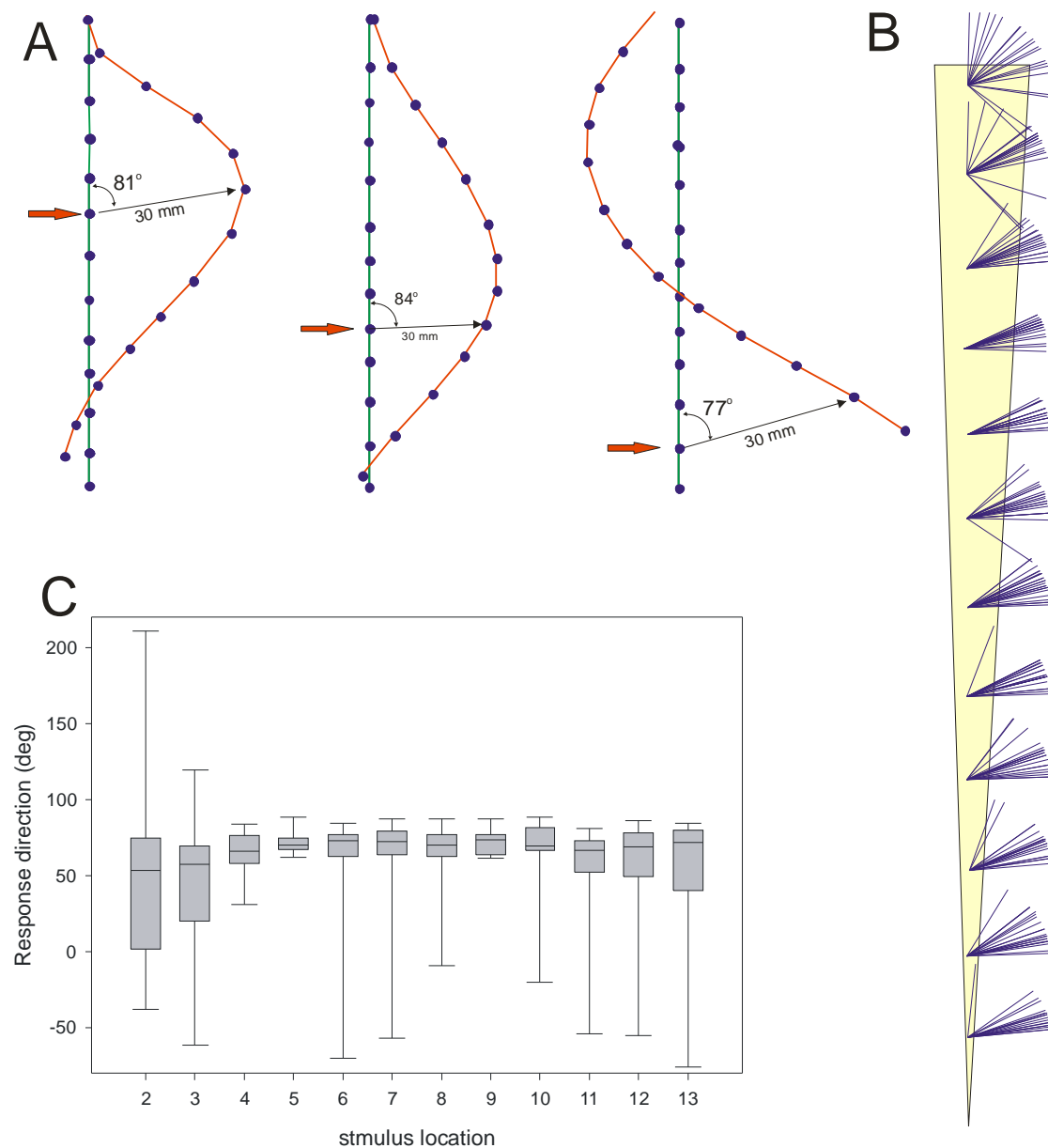


Figure 12. Response direction vs. stimulus location. (A) Three individual trials illustrating the response vector for stimulus locations 6, 9, and 12 (red arrow). The response direction at the stimulated location was measured from starting position to the position when it had moved 30mm (indicated with red). Response direction defined as the angle of the vector relative rostral (81°, 77°, and 84°). (B) Individual response directions for all trials at each stimulus location (n=247 overall; n=23, 19, 19, 18, 21, 22, 19, 22, 21, 20, 22, 21 for locations 2 through 13) showed almost uniform rostral-laterally directed responses. (C) Response direction did not depend on stimulus location (Watson – Williams P=0.48).

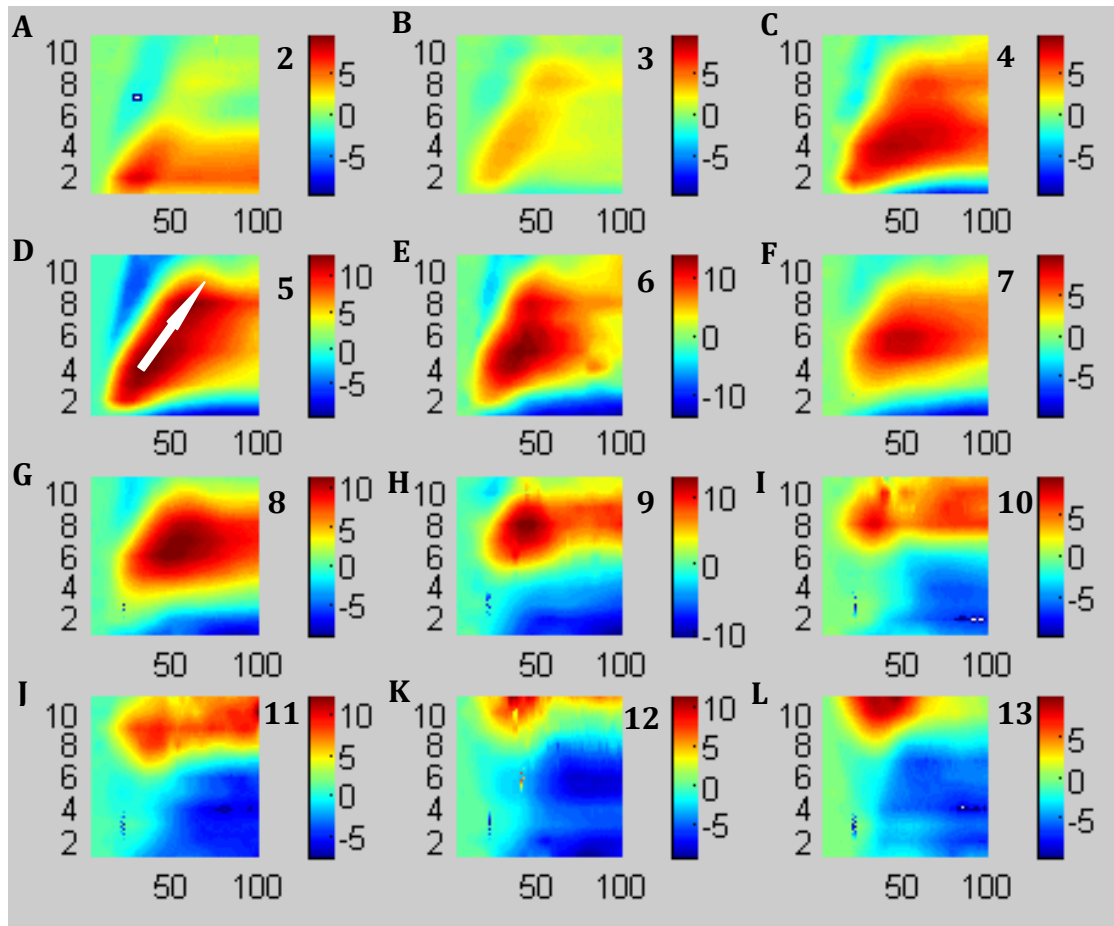


Figure 13. The local bend progresses distally over time. The contour diagram depicts angle of rotation (dark red indicates maximum rotation away from stimulus; dark blue indicates maximum rotation into the stimulus) for each stimulus location over time. Local bends correspond to peaks (or ridges) in angle (typically dark red), illustrated by the white arrow in panel D. Each contour diagram represents averages of 9 to 17 trials for each of the 12 stimulus locations, labeled by the number between the contour and the color map.

Curved, Rotated and Combined Postures

The second specific aim addressed by this study was the influence of initial tail posture on the kinematics of the NWR. As with the straight tail and regardless of initial posture, rotation at the base of the tail and a local bend near the stimulus location were observed for all (curved, rotated and combined) postures (Fig 14).

Rotation at the base of the tail reverses for extreme postures: Although rotation at the base of the tail was observed in all postures, the direction of rotation depended on the level of curvature and/or rotation. For example, for the straight tail and combined curved/rotated level 3 posture, the base of the tail, as expected, rotated away from the stimulus (Figs 15A, B and D). However for the most extreme combined posture (level 5), the direction of the base of the tail reversed and rotated into the stimulus (Fig 15C and D).

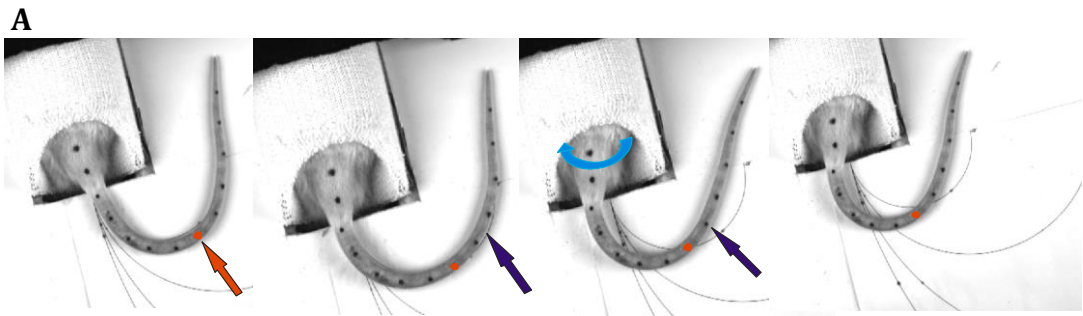
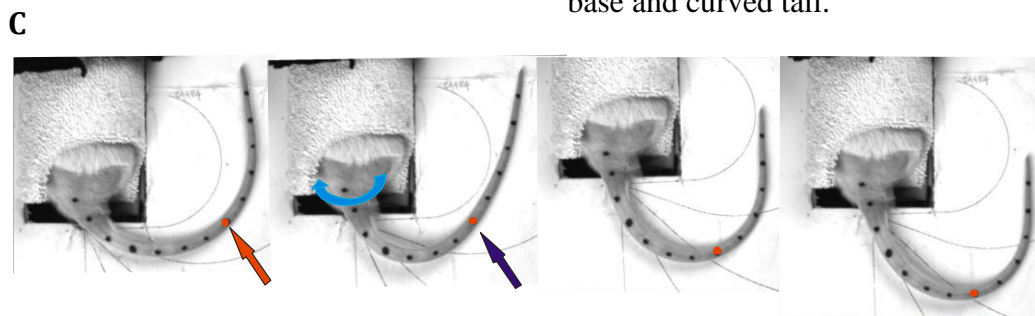
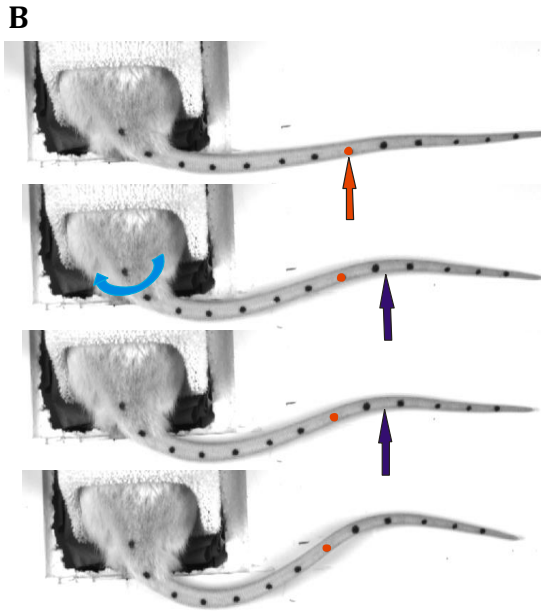


Figure 14. Representative examples of tail movement with different initial postures. Four video frames

(25ms, 15 frames apart) from a single trial for each posture show the movement of the curved, rotated, and combined tail in response to heat stimulation at location 8 (indicated by the red arrow). The blue arrow illustrates rotation around the base of the tail (rotation of location 2 relative to location 1) and the first purple arrow identifies the location of the local bend. The local bend (shown by the second purple arrow) progresses along the length of the tail as the movement continues. (A) Curved level 5 posture with the base of the tail straight (#1-2) (B) Rotated level 5 posture with the base of tail and tail straight (C) Combined level 5 posture with rotated base and curved tail.



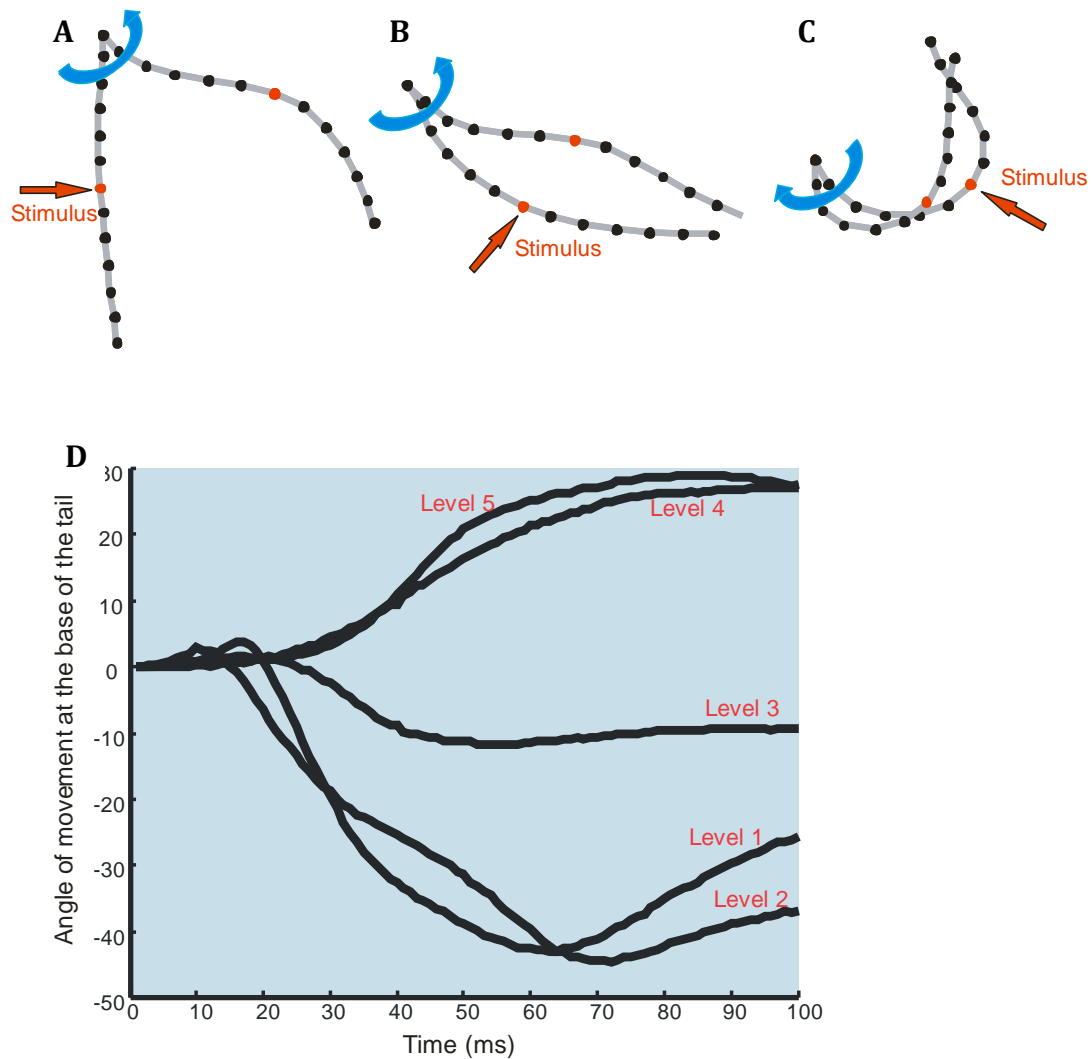


Figure 15. Representative example of the base of tail response for combined postures. The base of tail movement resulting from stimulation at location 7 (indicated with red arrow) for three levels of combined posture. (A) Straight tail (level 1) with stimulus delivered to location 7 caused the base of the tail to rotate away from the stimulus. (B) Combined posture (level 3); the stimulus caused the base of tail to again rotate away from the stimulus. Note the rotation was smaller than for the straight tail. (C) Combined posture (level 5) with stimulus again delivered to location 7 caused the base of tail to rotate in the opposite direction; into the stimulus. (D) Records of the angle of the base of the tail rotation over time for the trial shown above. For posture level 1, 2 and 3, the base of tail moved away from the stimulus (negative angle of movement) but for the more extreme postures level of 4 and 5, the base of tail moved into the stimulus (positive angle of movement).

In order to quantify the reversal in direction, tail base rotation was described by the frequency of the tail moving into the stimulus, maximum change rotation, and maximum angular velocity. Figure 16 shows the effect of posture (curved, rotated and combined), levels of posture (1-5), stimulus location (4, 8 and 12) and stimulus side (left or right) on each of these three measures. Only graphs with a yellow background were statistically significant.

For example, consider the rotated posture with left side stimulation for stimulus location 8. The bottom of three graphs shows that as tail rotation level increases, the frequency of movement into the stimulus progressively increases from 10% - 90% (χ^2 $P=0.0001$). The top graph shows that the magnitude of maximal rotation decreases as the posture is changed from level 1 to level 3 and then reverses and increases in magnitude for levels 4 and 5 (Friedman $P=0.003$). Similarly, the maximum velocity of rotation follows a pattern similar to magnitude (Friedman $P=0.004$). Together, all three measures of response show that as the tail was initially rotated to progressively more extreme angles, tail base rotation diminished and then reversed for the most extreme two initial changes in posture.

Tail base rotation was influenced by posture largely for stimuli delivered to the left side of the tail (left panels) rather than the right side (right panels), as demonstrated by the difference in the number of significant relationships (7 for left stimuli, only 1 for right stimuli).

Postures differed in their effects on tail base rotation. Curved postures had little effect (only one significant relation), while rotated (4 significant relations) and rotated/curved combined (2 significant relations) had a greater number of significant effects. In particular, stimuli delivered to location 8 were most frequently significantly influenced by posture.

Location of local Bend was not influenced by Posture: The relationship between stimulus location and posture was not influenced by any of the changes in initial posture. As shown previously with the straight tail (Fig 9 above), the location of the local bend matched stimulus location (four-way ANOVA $P < 0.0001$; $MC=6$; $\alpha=0.008$; effect size=0.83; Fig 17). However, neither the side (left or right; $P=0.88$) of the stimulus, level of posture (1 through 5; $P=0.42$) nor type of posture (curved, rotated or combined; $P=0.0004$; but small effect size=0.02) influenced the location of the local bend, as illustrated by the close similarity of the regressions lines in each of all six panels of Figure 14.

Magnitude of local bend was weakly influenced by posture: In contrast to the magnitude of tail base rotation, posture influenced seemed to have a weak the magnitude of local bend. Figure 18 compares straight tail posture and the most curved, rotated, and combined postures (level 5) to illustrate that the angle of local bend (angle between three green locations) is slightly smaller in all level 5 postures than straight tail posture (Fig 18).

Overall (Fig. 19), there was a barely significant progressive decrease in local bend angle with increasing levels of posture for rotated (Friedman $P=0.013$; $MC=3$; $\alpha=0.017$, significant; Kendall's $W=0.32$) and combined (Friedman $P=0.013$; $\alpha=0.017$, significant; Kendall's $W=0.31$) but not curved (Friedman $P=0.02$; $\alpha=0.017$, not significant) posture. The type of posture had no effect on magnitude of the local bend (two way ANOVA $P=0.78$).

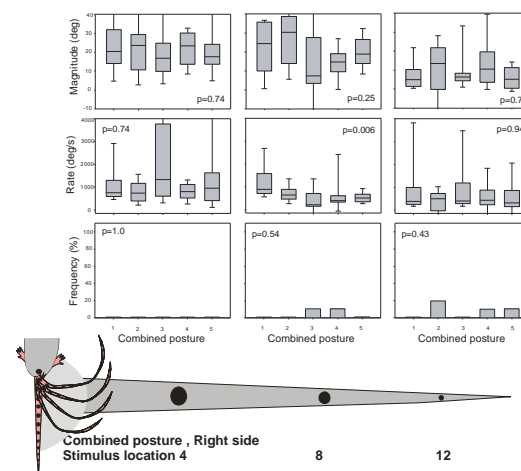
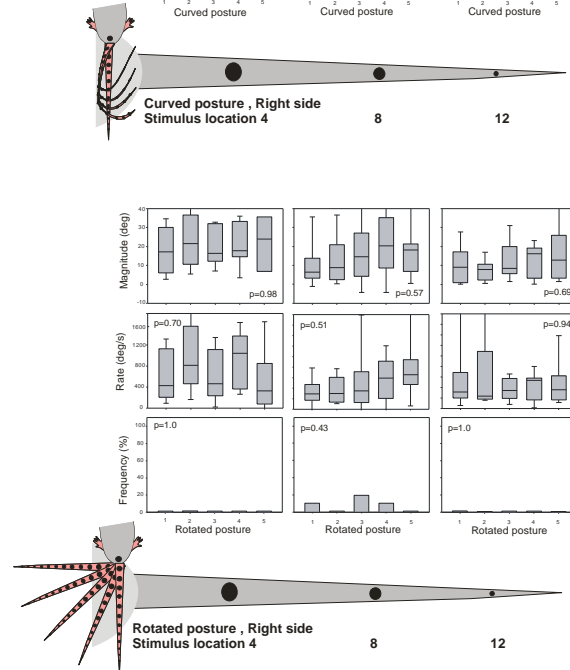
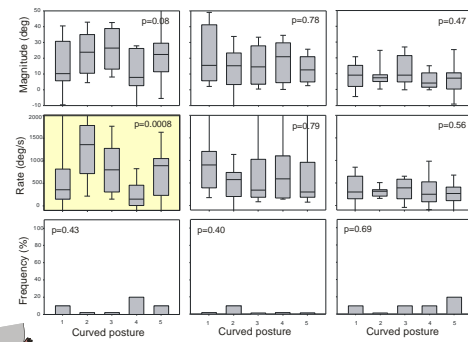
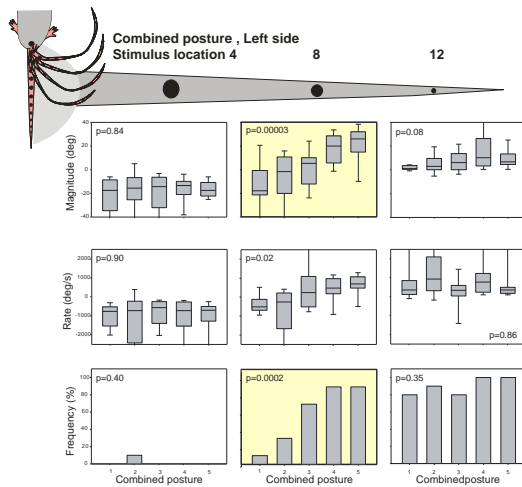
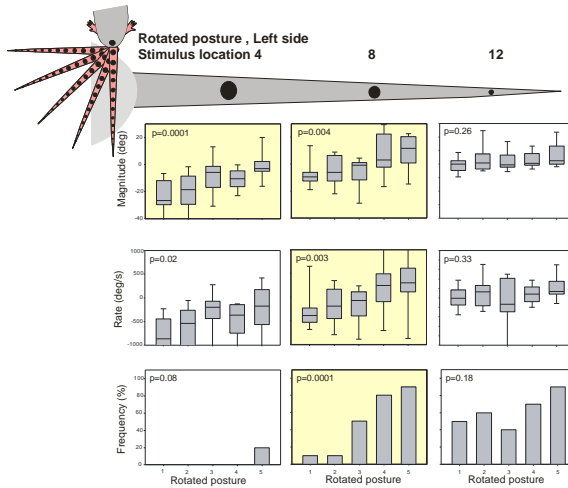
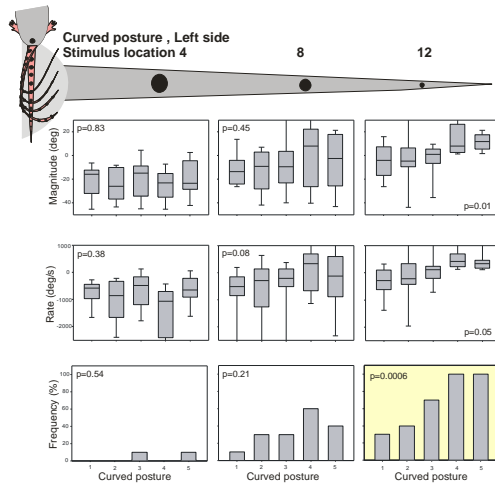


Figure 16. Dependence of tail base rotational movement on stimulus location and initial posture. Left three panels represent left side of tail stimulation of three initial postures and right three panels represent right side stimulation of three initial postures. Vertical 3 sub-panels correspond to different initial postures (curved, rotated, and combined postures). Within sub-panel, 9 graphs illustrate dependence of magnitude, rate, and frequency of tail moving into stimulus on the levels of posture. P-values are indicated within the graphs and significance are specified with yellow background (MC=9, $\alpha = 0.005$; n=900; Friedman and Kendall's W for magnitude and rate; W=0.49, 0.64, 0.39, 0.34, 0.23 (top to bottom, left to right); Chi² and Cohen's W for frequency; W=0.63, 0.68, 0.66 (top to bottom)).

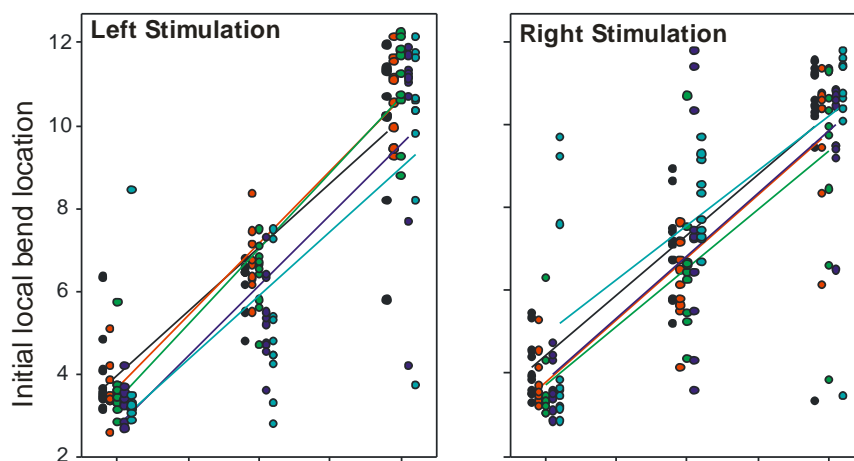
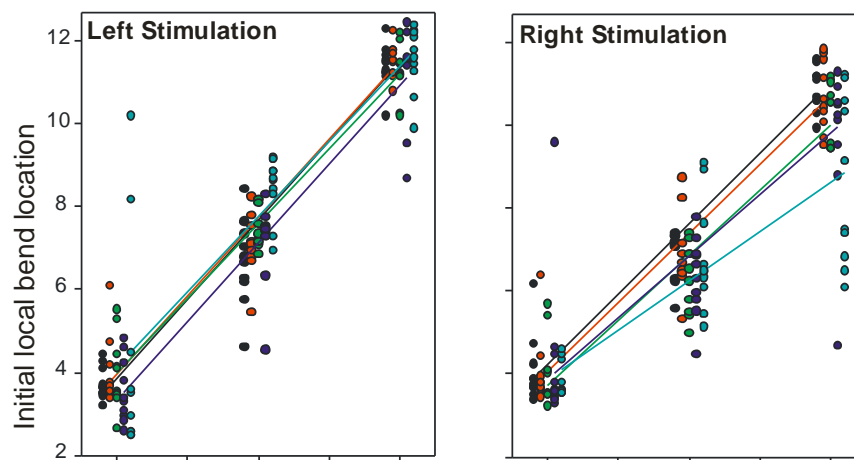
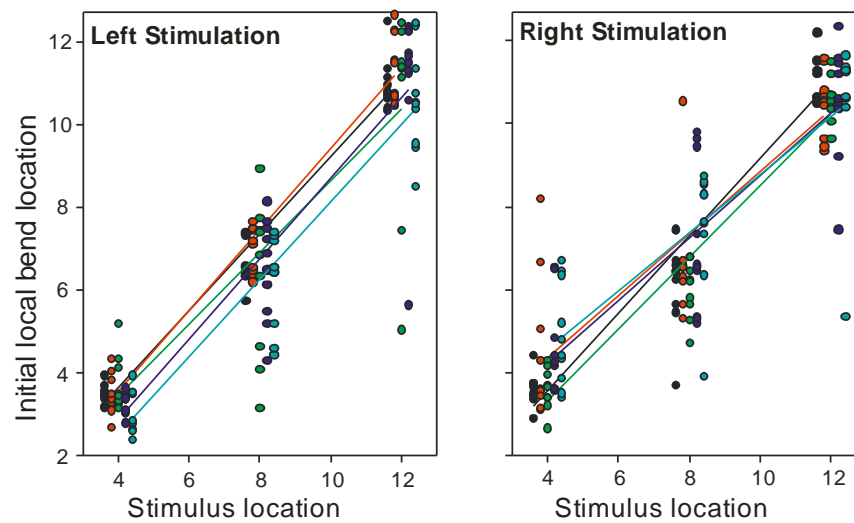
Curved**Rotated****Combined**

Figure 17. Stimulus location vs. initial local bend location. Within each graph, three vertical groups correspond to 3 stimulus locations (4, 7, and 11). The levels of postures are indicated by colors: (•) Level 1/straight; (•) level 2; (•) level 3; (•) level 4; (•) level 5. Each horizontal pairs of graph represents left and right side stimulation of curved, rotated and combined posture. Four – way ANOVA analysis showed that initial local bend location is significantly depended on stimulus location ($P < 0.00001$; $n=900$, effect size=0.83) while it was weakly significantly on type of posture ($P=0.0004$; effect size=0.02). Initial local bend location is not significantly depended on side of stimulation ($P=0.88$) and level of posture (0.42). Note that level of posture is 0.2 shifted in X-axis to help visualization.

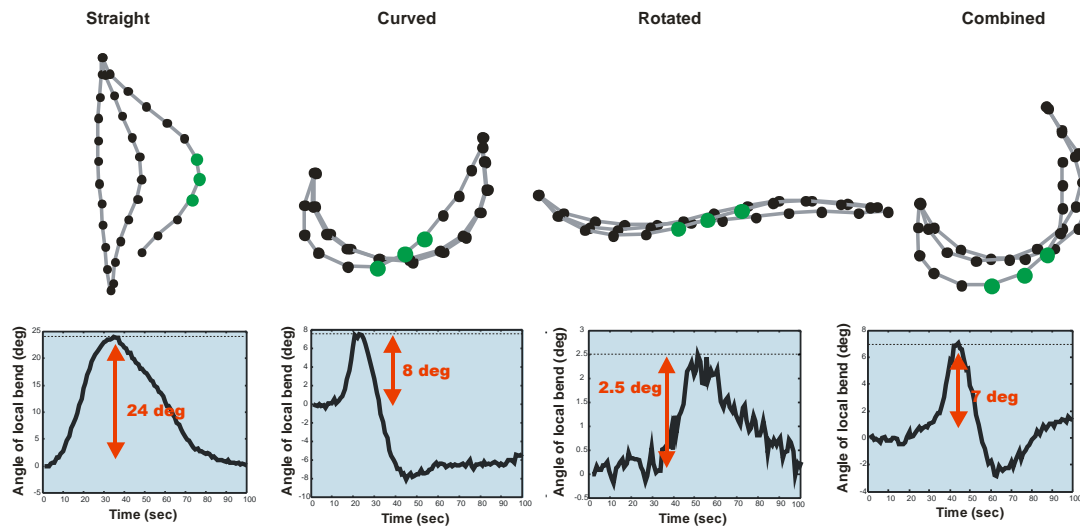


Figure 18. Change in magnitude of local bend at straight and level 5 of each initial posture. The variation of local bend magnitude was measured near the stimulus location (indicated with green) is observed in 3rd frames of straight and different initial postures (level 5). Local bend magnitude was measured by determining the peak angle of local bend near the stimulus location. Straight initial tail posture evoked local bend magnitude of 24 degree at the stimulus location. Curved initial tail posture (diameter 8 cm) with local bend magnitude of 8 degree at the stimulus location. Rotated initial tail posture (90 degree) with local bend magnitude of 2.5 degree at the stimulus location. Combined initial tail posture (diameter 8 cm and 90 degree) with local bend magnitude of 7 degrees at the stimulus location.

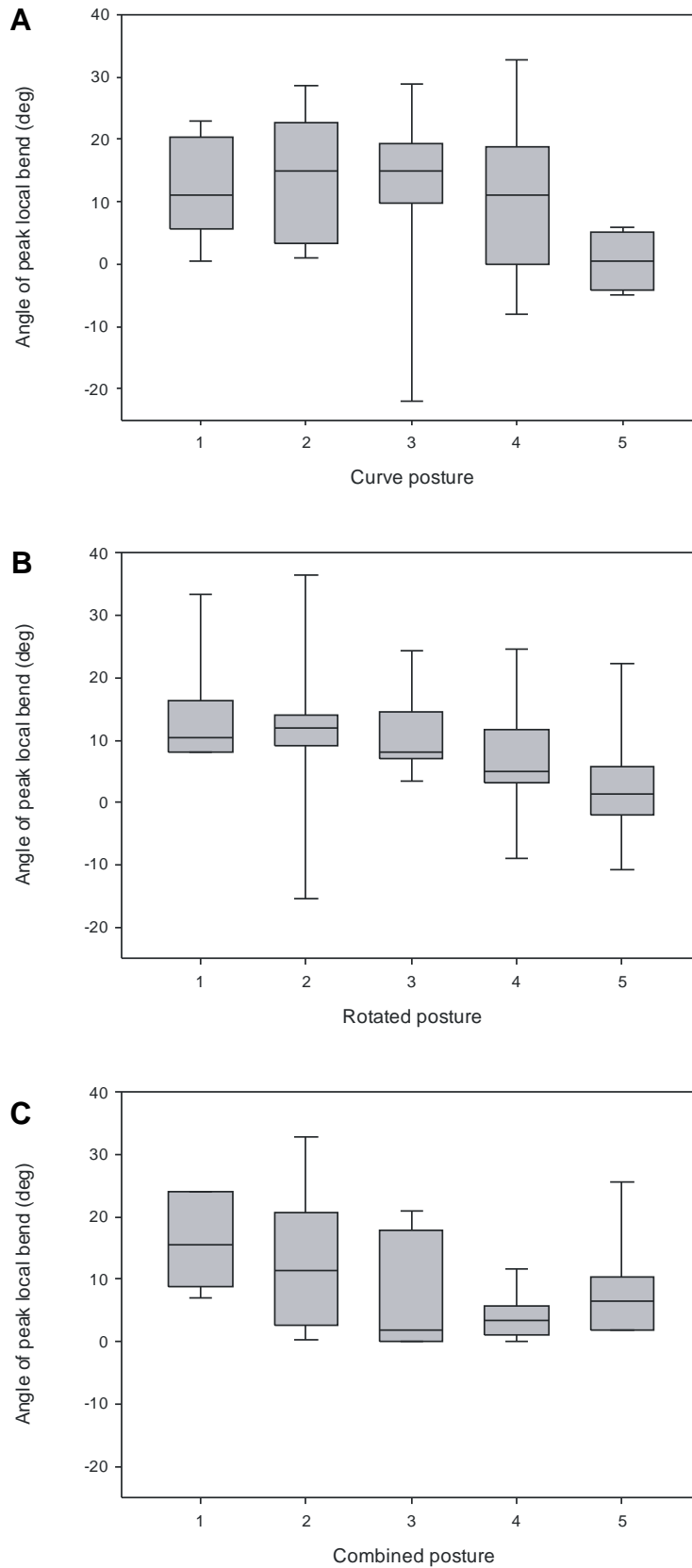


Figure 19. Change in magnitude of local bend at different levels at each posture and different initial postures. (A) For curved postures, the average angle of peak local bend did not depend on the level of curvature (Friedman $P=0.02$; $MC=3$; $\alpha=0.017$; $n=10$, not significant). (B) For rotated postures, the magnitude of peak local bend significantly decreased as the angle of initial rotation increased (Friedman $P=0.013$; $MC=3$; $\alpha=0.017$; $n=10$, Kendall's $W=0.32$, significant). (C) For combined postures, the magnitude of peak local bend significantly decreased as the angle of initial rotation increased (Friedman $P=0.013$; $MC=3$; $\alpha=0.017$; $n=10$, Kendall's $W=0.32$, significant). However, there was no significant differences between postures (Two – way ANOVA $P=0.78$).

Discussion

Summary of results

Previous studies conducted in spinalized, decerebrated, or anaesthetized animals have shown that the NWR may depend on the stimulus location and initial posture of limb. Therefore, the goal of this study was to determine the role of stimulus location and initial posture on the kinematics of the NWR of the rat's tail in *intact and unanesthetized* animals.

In response to noxious heat stimuli delivered to the left side of 12 different locations on tail, the NWR consisted of rotational movement at the base of the tail and a local bend near the stimulus site, resulting in a rostral-lateral movement of the stimulated location that was invariant in direction. The initial local bend location was slightly rostral to the stimulus location and the location of the local bend progressed caudally during the movement, consistent with passive mechanics.

When the initial posture was altered by rotation, but not curvature, the direction of rotation at the base of the tail reversed, changing from away from the stimulus to into the stimulus as the initial postural rotation level increased. Although the location of local bend was not influenced by the level and type of posture, the magnitude of local bend decreased as the initial posture level increased but the effect was small. These results suggest that the initial posture of the tail, specifically the initial rotation around the base of tail, plays a significant role in the NWR.

Straight Tail

Comparison with previous studies: The first goal of this study was to determine the effect of stimulus location on the kinematics of the NWR in intact and unanesthetized rats. This study showed that the NWR direction was invariant across all stimulus locations and that the responses were directed in only one direction: rostral-lateral (63.5°). However, there was a significant effect of stimulus location on the local bend; the location of bend was one location rostral to the location of the stimulus except at the most proximal locations.

Similar results were obtained from previous studies conducted in our laboratory in both spinalized (Cleland and Bauer, 2002; Bence, 2010) and intact (Cleland and Bauer 2002; Harrold 2009) rats. In intact rats, Cleland and Bauer (2002) and Harold (2009) also showed that the response to spatially diverse stimuli was largely invariant. In particular, stimuli that were distributed circumferentially around the tail at a single rostral-caudal location resulted in responses that were only in two directions – left-ventral and right-ventral. Stimuli to any location on the left side of the tail resulted in a right-ventral response while stimuli delivered to the right side resulted in a left-ventral response.

In spinalized rats, Bence (2002), using a similar experimental design to this research, produced strikingly similar results. The direction of response was nearly the same (64.3°) and the dependence of local bend on stimulus location was identical. These similarities suggest that these features of the response – direction invariance and close matching of stimulus and local bend – are mediated by the spinal cord.

Muscular mechanisms: Because the NWR was directed only in rostral – lateral orientation due to the combination of rotation movement around the base of the tail and local bend, it is important to consider whether or not the tail anatomy of the rat tail contributes to this result.

Tail base rotation and local bend may be mediated by lateral movement arising from two muscles in the pelvis (sacrocaudalis ventralis lateralis, SVL, flexor caudae longus, FCL) that have tendons inserting primarily from Co10 to Co28 (Hori et al., 2011), and eight large muscles in the pelvis (extensor caudae medialis, ECM; extensor caudae lateralis, ECL; sacrocaudalis ventralis medialis, SVM; Medial longissimus, ML; Abductor caudae dorsalis, ACD; sacrocaudalis dorsalis lateralis; Iliococcygeus, ICC) that insert tendons only into the base of the tail, between Co1 to Co10. Unilateral contraction of each of these eight muscles caused the tail to move laterally (Hori et al 2011, Brink and Pfaff, 1980). Because of their different insertions into the tail vertebrae, it is likely that SVL mediates lateral movement of the distal part of the tail (Co10-Co28) while ECM, ECL, SVM, ML, and ACD produce lateral movements of the base of the tail (Co1-Co10). Other extrinsic and intrinsic muscles are less likely to contribute the NWR (Hori et al., 2011; MacKenzie et al., 2013)

In support of this hypothesis, during the NWR evoked by heat, large EMG activities and tension were observed in the ML (largest), ACD, ECL (smallest; Cargill, 1983), which act on the base of tail. In contrast, the SVL muscle, which acts on the caudal portions of the tail, was not activated. These results are inconsistent with our observations of local

bends occurring at caudal locations for caudal stimuli since only the SVL could create the local bend. However, Cargill (1983) may have delivered heat stimuli only to rostral portions of the tail; thus activity in the SVL in our experiments would be expected to be absent, especially because movement caudal to the stimulus location is most likely passive (see below).

Passive versus active movement: Movement of individual tail segments can arise in two ways – actively and passively. Active movement occurs when the tendon of contracting muscles insert into the segment. Passive movement occurs when the movement of adjacent tail segments passively couples with the segment of interest. This distinction raised the question as to whether the observed movement of the tail – tail base rotation and local bend, as movement of the rest of the tail – arose actively or passively.

The lateral movement associated with the local bend is likely to arise actively because it occurs first and could not biomechanical arise from the movement of neighboring segments because they are moving less and later in time. The movement at the base of tail could arise either actively or passively. In support of passive movement, stimuli delivered to location near the base of that result in tail base rotation in the same direction as the lateral bend, which makes sense since the rostral segments of the tail are stiff (Ben Cornelius, unpublished observations). In contrast, stimuli delivered to the caudal segments of the tail resulted in tail base rotation that was opposite to the direction of local bend, which is consist with the distal segment being more flexible.

Finally, the observations that the bend progresses caudally suggest that movement of the tail caudal to the stimulus location may be passive because similar bend progression occurs in strictly mechanical systems such as fishing rods (Wang and Wereley, 2011) and whips (McMillen and Goriely, 2003). However, active movement may also contribute, such as in the octopus (Gutfreund et al., 1996).

Curved, Rotated and Combined Postures

Comparison with previous studies: The second goal of this study was to determine the effect of initial posture on the kinematics of the NWR in intact and unanesthetized rats. This study showed that the initial posture of tail has significant effect on the direction, rate, and magnitude of rotational movement around the base of tail but either weak or no significant effect on the magnitude and location of local bend.

Our results, which showed that tail base rotations reverses in direction for rotated postures is broadly consistent with previous findings by in human and animal models (Baxendale and Ferrell, 1980; Peterson et al., 2013; Serrao et al., 2006; Kim et al., 2007). In particular, previous studies in our laboratory (Chrzan, 2013; Seamon, 2015) showed that in the intact and anaesthetized rat the NWR of the foot depended on initial posture. That is, when the foot was initially placed by the rat relatively forward, the NWR caused it to move backward and when the foot was initially placed backward forward, the NWR caused it to move forward (and similar for left and right). Thus in both series of experiments, the rat tended to move it tail back to a “reference” position; straight for the tail.

Previously in our laboratory, the relationship between initial posture of limb and NWR was studied. Although, results of those studies were measured by tracking initial and final position of paws instead of tracking the entire movement of the tail NWR, both studies showed that if rat's paw was rostral, it moved caudally and if the paw was caudal, it moved to rostral. These results showed that there is a significant relationship between the direction of the NWR and initial paw position, which is similar to the result of this study where the direction of the tail base decreased and reversed as the level of posture increased.

In contrast to the broad dependence of the NWR on posture from previous studies (Baxendale and Farrell, 1980, Kim et al., 2007, Peterson et al., 2013), this study found that the location of the local bend is independent of the initial posture and that the magnitude of the response is only weakly influenced. There are several possible explanations for the differences between our work and previous work. First, the model systems differed. Most previous studies used spinalized animals while we used intact and unanesthetized animals. Also, previous studies focused on the NWR of limb while we focused on the NWR of tail, which differ structurally and functionally. More intriguingly, however, since the local bend may arise from spinal mechanism (discussed earlier), it is possible that supraspinal but not spinal mechanisms are influenced by initial posture.

Biomechanical mechanisms: In contrast to the straight tail, when the initial postures are altered we observed that the direction of rotation around the base of the tail reversed. Reversal could be due to two possible mechanisms: muscular biomechanics or proprioceptor feedback

When the initial tail posture is altered to the most extreme postures (level 4 or 5), the force vector component – from the perspective of the tail segment – are identical. However, the tail-oriented rostral-caudal force vector is now directed to the left rather than rostral. Consequently, the net torque around the base of the tail may reverse (Fig 20). However, the occasional (8.4%) finding that the straight tail moves into the stimulus argues against a strictly mechanical mechanism.

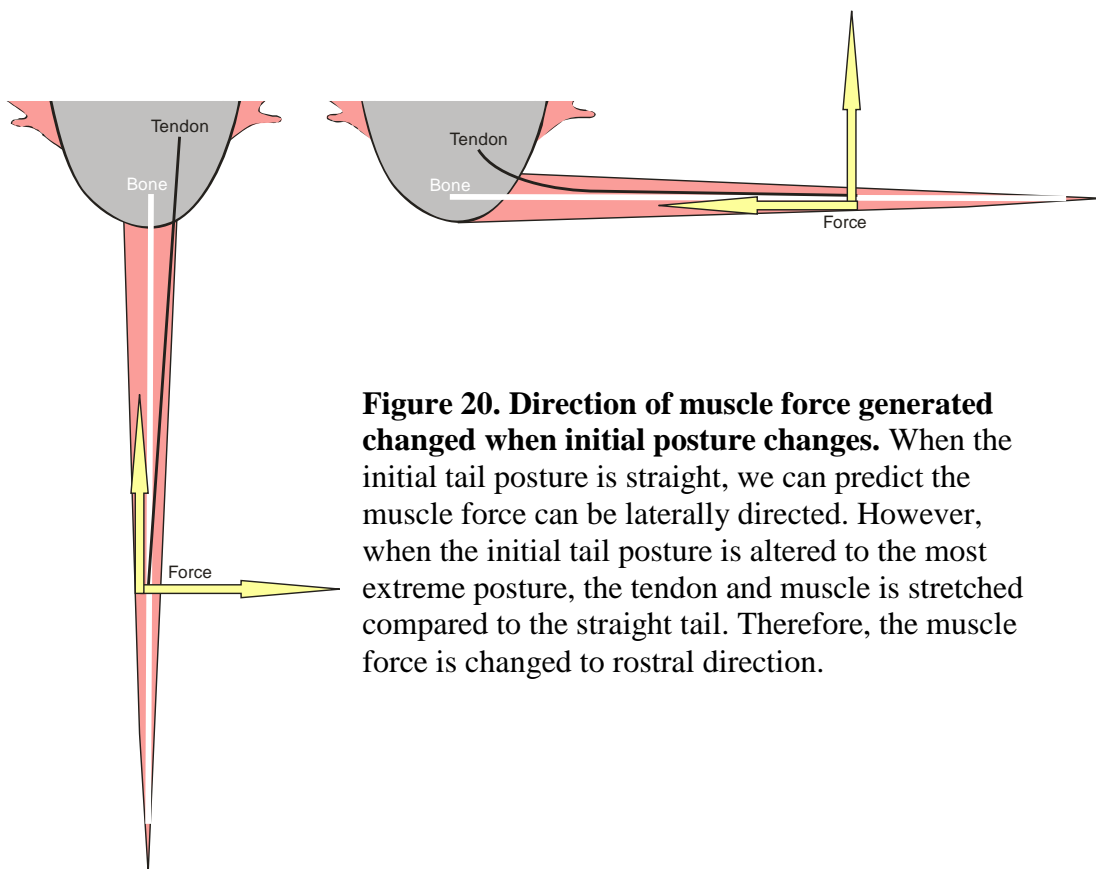


Figure 20. Direction of muscle force generated changed when initial posture changes. When the initial tail posture is straight, we can predict the muscle force can be laterally directed. However, when the initial tail posture is altered to the most extreme posture, the tendon and muscle is stretched compared to the straight tail. Therefore, the muscle force is changed to rostral direction.

Alternatively, the change in direction could be due to proprioceptor feedback altering the neural commands. There are three proprioceptors in tail: muscle spindles, Golgi tendon organs, and joint receptors. The activity of muscle spindles encodes muscle length while

Golgi tendon organs encode muscle forces. Joint receptors encode the angle, direction, and velocity of joint movement changes (Proske and Gandevia, 2011). In the rat's tail, muscle spindles are distributed throughout the tail (Thompson, 1970) and therefore may feedback provide information on tail posture.

There is evidence that changes in hip joint angle, acting through hip proprioceptors, influence knee and ankle stretch reflexes (Onushko et al., 2013). The stretch reflex latency decreased significantly when the posture changed from standing to supine position (Pope and DeFreitas, 2015). While these studies showed that changes in angle or postures of limbs could affect the non-nociceptive reflexes, it is possible that the NWR of tail was also influenced by proprioceptive feedback from the tail.

The observation that postural rotation at the base of tail but not tail curvature results in reversal of tail base rotation suggests that tail base proprioceptors, most likely spindle or joint receptors, are responsible. This seems to be consistent with previous studies showing the importance of hip, but not knee or ankle, proprioceptors for modulation of locomotion (Pang and Yang, 2000)

Synthesis: Model for Tail Movement

The kinematics of the NWR suggests a potentially simple kinetic model for the NWR. Following stimulation, muscle(s) apply rostral-lateral force directly to the segment being stimulated, which results in an active local bend, passive rotation of the more proximal tail base and passive “whipping” of the distal regions of the tail. When the initial posture

of the tail is altered by rotating the tail to the side, the reflex rotation around the tail base reverses, thus moving the tail into the stimulus but away from the body. This change in direction may arise just passively from the altered posture. Taken together, although the kinematics of the tail movement are complex, these results suggest that the rat may use a simple kinetic strategy, based solely on applying force only to the location stimulated, to effectively withdraw its tail from noxious stimuli under various postural conditions.

Significance

Mammals, including humans, make both purposeful and automatic movements. However, completing those tasks rapidly, accurately and in coordination with the body and environment can be difficult because mammals have large number of typically redundant joints DOF. In order to overcome this complexity, mammals can use kinematic synergies, re-use higher level motor commands and select preferred movements. The present study showed that rats decreased the number of DOFs by limiting their tail movement to rotation around the base of tail and local bend (kinematic synergies). In response to the heat stimuli to different locations, rats withdrew their tail only in direction, rostral-caudal, regardless of stimulus locations (re-use of higher level commands). Lastly, rats reversed the direction of rotational movement around the base of tail as the initial postural rotation level increased (select preferred movement pattern). These movement strategies both potentially simplify the neural control of movement while still effectively matching the movement to both stimulus location and posture.

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